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On the Acoustic System in the Cetacean Brains

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Introduction

It was first pointed out by SPITZKA (1886) and a little later by ZIEHEN and KÜKENTHAL (1893) that the acoustic system in the brains of the Cetacea is very well developed. HATSCHEK and SCHLESINGER (1902) referred to it in their study of the brain stem of a Delphinus. Later, HOFMANN (1908) investigated the superior olive of a few kinds of dolphins and the inferior colliculus was studied in the same group of animals by VALETON (1908). Recently LANGWORTHY (1932) examined the whole brain of Tursiops truncatus both macroscopically and microscopically, concluding that its acoustic system is in a high stage of development. It should be noted here that only the Odontoceti, mostly the dolphins, have been studied so far in this respect and scarcely any reference has ever been made to the acoustic system of the baleen whales, nor has there been any comparative study between Odontoceti and Mystacoceti regarding this system.

One of the reasons which prompted us to our present study is, apart from the comparative anatomical interest, the desire to estimate the auditory sense of the sperm whale and various Mystacoceti, at least as far as it is possible through the study of their brain stems. We are sure it is of practical importance to know about the sharpness of hearing in those whales, which are the chief objectives of the present-day whaling enterprise.

Six kinds of whales are studied in our present work: of the dolphins, Lagenorhynchus obliquidens GILL and Tursio borealis PEALE, of other Odontoceti, Kogia breviceps BLAINVILLE (pigmy sperm whale) and Physeter macrocephalus LESSON (sperm whale, cachalot); of the Mystacoceti, Balaenoptera borealis LESSON (sei whale) and Balaenoptera acuto-rostrata LACÉPÈDE (little piked whale). Serial sections of the brain stems of these six animals, all stained by the PAL-carmin method, have been examined with the object of studying the structures in the acoustic system, of determining the difference between Odontoceti and Mystacoceti with references to the

acoustic system of other mammals.

The Lagenorhynchus in our present study is an embryo of about 70 cm length, which corresponds, judging by the degree of myelinization in its brain stem, nearly to the newborn human infant. The Tursio is an embryo of about 45 cm length, whose brain stem is in the same stage of development as that of 7 months old human embryo. In this Tursio embryo the myelinization of nerve fibers belonging to the acoustic system has just started, while most of other fibers in the brain stem remain as yet unmyelinated. The very rare brain of Kogia breviceps was collected by T. Ogawa at Shiogama, Miyagi Prefecture on June 7th, 1936. The details on this specimen were already reported in "Botany and Zoology" Vol. 7, No. 7 (published by Yokendo, Tokyo. July, 1938). The brain stem of Physeter macrocephalus is not in perfect condition and the serial sections are limited only to the levels from the oral part of pons to the caudal part of mid-brain. Serial sections of the brains of two Mystacoceti are complete, in Balaenoptera borealis in transverse section and in B. acuto-rostrata in sagittal section. All the brain stems of four Odontoceti are sectioned transversely.

The details of our acquisitions will be reported later in another paper, and only summary of our work is presented here due to the limitation of space.

Results (summarized)

1. "Telescoping" of the dolphin's brain stem.

One of the most characteristic features in the brain stem of dolphins is the caudal shift of its dorsal structures. This peculiarity is not so apparent in the caudal part of oblongata, but on the level near the caudal end of pons, the axis of the brain stem seems to bend dorsally, presenting a curious topographical relation, as in one transverse section of the brain stem one sees in the ventral part structures belonging to the oral proximity of oblongata, in the dorsal part those of mid-brain and in the middle part structures corresponding to the pontile level.

Strangely enough, this unusual feature seems to have been overlooked by many researchers who have studied the brain stem of dolphins. Either no notice has been taken of it, or it was simply dismissed as a result of oblique section. As a matter of fact, however, this is very important in

evaluating the structures in the brain stem of dolphins, and we would like to emphasize it.

In 1923 MILLER described a phenomenon called "telescoping" of the Cetacean skulls. According to him, the maxillary bone as well as the frontal bone is elongated caudally, one over the other, coming in contact with the occipital. The above mentioned shift in the brain stem of dolphins seems to have an intimate relation with this unusual configuration of the skull and we wish to use the term "telescoping" also for the brain stem of dolphins. Meanwhile it is as yet impossible for us to decide whether or not the telescoping of dolphin's skulls is directly responsible for the telescoping of the brain, as no remarkable shift of structures is to be seen in the brain of other whales such as Kogia, Physeter and Balaenoptera, even though MILLER'S phenomenon is also present in the skulls of these animals.

The brain stem of dolphins is narrow from side to side and broad in ventrodorsal direction. This is also one of its characteristics.

LANGWORTHY said that the whole brain of Tursiops is foreshortened in its anteroposterior extent and widened laterally due to change in the conformation of the skull. This is true, in our opinion, as far as the cerebral hemispheres are concerned, but in the brain stem, especially in its caudal part, the transverse dimension is remarkably small. It is of interest to note here that the entire oblongata in dolphins, when considered in its topographical relation to the skull, seems to be caudally shifted, as both cochlear and vestibular roots run orocaudally to reach the brain stem in its lateral part. The cerebellar flocculus surrounds the ventrolateral surface of oblongata and pons, and the nucleus ventralis of the cochlear nerve lies buried deep between flocculus and the brain stem. The cochlear root approaches the brain stem obliquely and enters the oral part of the ventral nucleus.

2. Dolphins and sperm whales.

Though both dolphin and the sperm whale including Kogia, belong to the Odontoceti, many differences are observed in their acoustic systems, in all probability due to the above mentioned peculiar form of the brain stem at the side of dolphins. There is nothing, however, to indicate the existence of an essential difference between them.

In the case of Kogia, the ventral nucleus of the cochlear nerve pro-

trudes from the ventrolateral part of the oral end of oblongata. Its caudal half is ventrally exposed to the view owing to the absence of flocculus over its ventral surface, while its oral half lies covered by the fibers of brachium pontis. The cochlear and vestibular nerves enter the nucleus in its medial part, as is generally the case in the mammals. The ventral nucleus is as well developed as that of Lagenorhynchus, and it is hard to decide which is more prominent.

In Odontoceti, the corpus trapezoides, which emerges medially from the ventral nucleus, is in a very good state of development; its transverse fibers occupy in Lagenorhynchus more than half (60%) of the dorsoventral length of the raphe, and in Kogia, a little less than the half (43%). The same applies to HELD'S tract, which runs quite separated from the trapezoidal body and joins this in the median line, where it forms the dorsal part of the trapezoidal body.

Massive fibers emerge from the dorsal half of the ventral nucleus in the fetal dolphins, seemingly ending in the superior olive of the same side. In Kogia it is hard to distinguish this group of fibers, probably because its myelinization is in a far advanced stage. In his study of myelinization in the cochlear system of man, dog, cat, rabbit and rat, one of us (OGAWA, 1936) observed the same kind of homolateral trapezoidal fibers as in the fetal dolphins.

Noteworthy difference is found regarding MONAKOW'S striae acusticae. Those of Lagenorhynchus consist of a great number of fibers, which form a compact bundle, while in Kogia they are made of much fewer fibers. This should not be interpreted as an essential difference, because MONAKOW'S tract also arises from the ventral nucleus of the cochlear nerve and is nothing but a part (pars dorsalis) of the trapezoidal body. In the same way HELD'S tract should be considered as its intermediate part. In Odontoceti no trace of the dorsal nucleus of the cochlear nerve, i. e. tuberculum acusticum, was observed.

One of the most impressive features in the brain stem of Odontoceti is the remarkable development of the superior olive. We need not enter into details about this nucleus of dolphins, as it is fully described by HOFMANN, who studied it in Phocaena communis and Delphinus delphis, and also by LANGWORTHY, who investigated that of Tursiops truncatus.

Partition of superior olive in two parts, pars lateralis and pars medialis,

is hardly recognizable in Lagenorhynhus. It is almost the same with Tursio. Immature myelinization of the fetal brains is probably responsible for it. The superior olive of Kogia is relatively larger than that of dolphins. In fact no larger superior olive can be found in all the whales we have investigated. Distinctly divided in two parts, the pars lateralis is disproportionately bigger than the pars medialis. The nucleus as a whole presents a very light colored aspect, as there are relatively few myelin-stained fibers within it. The pars medialis is much smaller, its oral end lying more backward than that of the pars lateralis. It should be noted here that this caudal position of pars medialis is an exception to HOFMANN'S conclusion in his comparative anatomical study of the superior olive, inasmuch as he says that in many animals he studied the pars medialis is invariably found to be on a more oral niveau than the pars lateralis.

The trapezoidal nucleus is very well developed both in Kogia and in Lagenorhynchus, with only the following difference: in the latter, probably owing to the narrowness of the brain stem this nucleus borders on the median line, while in the former, there is some space between the nucleus and the raphe. Needless to say, this does not mean an intrinsic difference. A part of the trapezoidal nucleus is found wedged between the superior olive and the lateral lemniscus, protruding into the dorsomedial part of the latter. This is common to both of the Odontoceti.

One of the similarities between Kogia and dolphins is the vastness of the lateral lemniscus. That it is very large in dolphins has already been known, but in Kogia and Physeter it is strikingly larger. The term "colossal" can safely be used in describing it. One is easily led to imagine a malformation of the brain stem. The nucleus of the lateral lemniscus and the inferior colliculus with its brachium are also proportionately large. The medial geniculate body is completely merged in the posterior part of the optic thalamus, and even though its hugeness can easily be surmised, we were unable to measure its actual size.

Generally speaking, much similarity is observed in the acoustic system of Kogia and dolphins. Remarkable development of the ventral nucleus of of the cochlear nerve, corpus trapezoides, HELD'S tract, trapezoidal nucleus, superior olive, lateral lemniscus with its nucleus and the nucleus colliculi caudalis is quite unique among all the mammals. MONAKOW'S striae acusticae are more prominent in dolphins. Kogia and Physeter may well

be said to surpass dolphins in the development of the superior olive, the lateral lemniscus and the inferior colliculus.

3. Odontoceti and Mystacoceti.

In the topographical relation of a great many structures in the brain stem, Balaenoptera has much in common with Kogia, presumably because there is no telescoping in their brain stem. At first sight, it seems as if Balaenoptera is more akin to Kogia than to Lagenorhynchus in their acoustic systems. Further investigation, however, reveals the existence of an intrinsic difference between them.

The ventral nucleus of the cochlear nerve and the corpus trapezoides are very well developed in Balaenoptera. The latter occupies in Balaenoptera borealis 40% of the raphe and in B. acuto-rostrata 47%, coming very close to that of Kogia (43%). In Balaenoptera there is not a vestige of HELD'S tract and MONAKOW'S striae acusticae are poorly developed.

The most significant difference is observed between Balaenoptera and Odontoceti in the development of the superior olive. That of B. borealis is very small, and it looks very dark as it has many myelinated fibers among the nerve cells. The pars lateralis of the superior olive in Kogia is disproportionately large compared with the pars medialis, or with the pars lateralis of Balaenoptera. That of B. borealis appears on the oral one third of the facial nucleus, terminating on the level of the oral proximity of this nucleus (orocaudally 4 mm, transversely 4 mm), while that of Kogia begins on the middle level of the facial nucleus and reaches the transitional part of the superior olive to the lateral lemniscus (orocaudally 5 mm, transversely 6 mm). The pars medialis of the superior olive in Kogia is small and disappears on a more caudal level, while in Balaenoptera it is fairly long in orocaudal direction, outstripping its pars lateralis by 4.5 mm. Histological difference of the superior olive between Odontoceti and Balaenoptera seems also to be of importance. As has been mentioned before, the superior olive of Kogia presents a very light colored aspect, as there are relatively few nerve fibers among its cells, while in Balaenoptera it is very dark on account of abundant fibers within the gray substance.

Meagre development of the superior olive in Balaenoptera in spite of its large trapezoidal body seems to indicate that a great number of the latter's fibers reach the lateral lemniscus without interruption, relatively a small part of it terminating in the superior olive. It is also conceivable

that some of the trapezoidal fibers come directly from the cochlear root and end in the trapezoidal nucleus. This nucleus is fairly well developed in Balaenoptera but not so well as that of Odontoceti. A small part of the trapezoidal nucleus, which lies in Odontoceti between the superior olive and the lateral lemniscus, is not observed in Balaenoptera. The lateral lemniscus is as well developed as in most other mammals but falls short of Odontoceti in its size. The inferior colliculus of Balaenoptera with its brachium is above the average of mammals in its development but poorer in comparison with that of Odontoceti. The medial geniculate body is fairly large, but its inner structure does not seem to be in good differentiation, as we see neither any trace of an incisure on its lateral surface, nor any difference between the peripheral and the deeper regions in its ventral part.

Considered as a whole, there is a remarkable difference between Mystacoceti and Odontoceti in the development of the superior olive and the lateral lemniscus. We are not at present in a position to say what functional meanings these structures have in the auditory sense, but judging from the degree of development of intracerebral structures in the acoustic system, we may assume that Odontoceti must be more acute of hearing. In this respect Mystacoceti seem to be inferior to Odontoceti.

4. Man and Balaenoptera.

Inferiority of the acoustic sense of Balaenoptera to that of Odontoceti does not necessarily imply the bluntness of hearing in the former, as structures of the acoustic system developed in Balaenoptera are by no means below the average of mammals. Balaenoptera bears at least one resemblance to man in that the superior olive is very small compared with the well developed trapezoidal body. In this respect there seems to be something in common between Homo and Balaenoptera in their auditory sense. At the same time one should not forget the fact that Balaenoptera's superior olive as well as its trapezoidal body is in a slightly better state of development than that of man and the trapezoidal nucleus occupies a large area, while in man this nucleus is hardly recognizable.

5. The corpus ponto-bulbare of Essick, the dorsal nucleus of the cochlear nerve and the dorsal nucleus of the lateral lemniscus.

The corpus ponto-bulbare, first fully described by Essick, is hardly known to exist except in the human brains. Certainly there is no trace

of it in dolphins, but in Kogia it is very well developed and is also distinctly recognized in Balaenoptera. When in 1933 WILSON described the brain stem of Balaenoptera borealis, he mistook this gray matter for the ventral nucleus of the cochlear nerve (Journ. Comp. Neurol. Vol. 58, No. 2, p. 455, fig. 18), and the space left blank just lateral to "tpz" in his illustration also belongs to it. The fibers intimately related to the ponto-bulbar body are attached to the lateral and the medial sides of the ventral nucleus both in Kogia and Balaenoptera. In B. borealis, these fibers run dorsalward parallel to the corpus restiforme, forming the most lateral of three bundles directly lateral to this body. Each of these bundles seems to have a significance of its own.

In the study of the acoustic system of whales, due attention should be paid to the existence of corpus ponto-bulbare.

The existence of the nucleus dorsalis nervi cochleae, i. e. tuberculum acusticum, in whales is very doubtful. We could not locate it in Odontoceti and found only a rudimentary one in Balaenoptera. According to FUSE, a partial fusion of the dorsal nucleus of the cochlear nerve to the ventral nucleus occurs sometimes in higher animals. In this connection the question arises whether or not the pars dorsalis of the ventral nucleus we observed in dolphins is to be considered as the dorsal nucleus itself. But our reasons for assuming the non-existence of the dorsal nucleus in Odontoceti are as follows.

In dolphins the ventral nucleus is divided in two parts, pars dorsalis and pars ventralis, while in Kogia it is divided in pars lateralis and pars medialis. The fact that the dorsal and ventral parts of the former correspond respectively to the lateral and medial parts of the latter is out of question, as the cause of this topographical difference lies certainly in the general form of the brain stems. In dolphins the brain stem is broad in ventrodorsal direction and narrow transversely, while in Kogia it is quite the opposite. Had the dolphin's brain stem been shaped just like that of Kogia, the ventral nucleus of the cochlear nerve might have been divided in pars lateralis and pars medialis as in Kogia. As it is, with its topographical relation to the cerebellar flocculus and to MONAKOW'S striae acusticae being duly considered, we cannot identify in dolphins the dorsal part of the ventral nucleus with the dorsal nucleus of other mammals.

Next we wish to call attention to the so-called dorsal nucleus of the lateral lemnicus, which is located both in Kogia and Lagenorhynchus, just ventral to the nucleus of the inferior colliculus and seems to be closely connected with "fibrae perforantes". This nucleus remains very small among all other very well developed cochlear structures, and so it is very doubtful that this tiny gray matter has anything to do with the cochlear system. Probably it has some other functional significance. In Balaenoptera no trace of this nucleus is to be seen.

VALETON, in her comparative-anatomical study of the inferior colliculus, found a fiber group of small calibre in the lateral lemniscus of Phocaena communis emerging from its dorsomedial part. Some of it are believed to reach as far as the contra-lateral inferior colliculus by way of velum medullare anterius and some of it to run dorsally along the medial margin of the inferior colliculus until they disappear near the commissure of the inferior colliculus. Described in details as they were, we can not in any way locate such a fiber group in Lagenorhynchus. Some finely myelinated fibers are observed by us to arise out of the dorsomedial part of the lateral lemniscus in Lagenorhynchus. These fibers either pass through the brachium conjunctivum or over it to reach the central gray matter, where most of them disappear with only a small portion of them reaching as far medial as the surface of the fourth ventricle. They represent no doubt VALETON's fibrae marginales (loc. cit. p. 30 a. 59). In cachalot we saw several thick bundles of the same calibre pass through the brachium conjunctivum and reach the central gray matter dorsal to the fasciculus longitudinalis medialis, where they bend suddenly ventralward. In Kogia the existence of such fibers is not so clear, while in Balaenoptera borealis a thick bundle goes into velum medullare anterius ofter crossing the trochlear root. We could not decide whether or not this bundle has anything to do with the acoustic system.

6. Nuclei of the vestibular nerve, especially the DEITERS' nucleus.

In all the cetacean brains studied here, meagre development of the vestibular nuclei as a whole stands out in striking contrast to the very well developed cochlear nuclei. In Lagenorhynchus, the vestibular root is barely one tenth of the cochlear one in thickness.

In studying the vestibular system of whales, due consideration should be given to the existence of a part of the vestibular root, so-called tractus

solitarius acustici of LEWANDOWSKY, which runs, apart from the main tract, dorsally along the lateral margin of the restiform body. The cells of the DEITERS' nucleus lie scattered along this root, a few of them reaching as far ventral as the ventrolateral margin of the restiform body.

The nucleus triangularis of Kogia and Balaenoptera occupies a smaller area than that of Lagenorhynchus, as in them the central gray matter of the fourth ventricle is more limited in space. This fact, however, should not be considered as showing some definite difference in vestibular function, as the nucleus triangularis is not limited to the vestibular nerve in its function.

The spinal tract of the vestibular nerve is very meagre in Lagenorhynchus and its fiber bundles are in a looser arrangement. In Kogia and Balaenoptera this tract occupies a far wider area and its fibers are massively collected. Except in Lagenorhynchus, the BECHTEREW'S nucleus is hardly recognized in whales, a finding quite contrary to KAPLAN'S, who found this nucleus very well developed both in Phocaena and Balaenoptera. Incidentally it should be mentioned that according to KAPLAN, this nucleus in whales is the shortest one of all animals in orocaudal direction, though it occupies a broad area on a transverse section.

In our opinion, of all the vestibular nuclei only the DEITERS' nucleus is very well developed in whales. In Lagenorhynchus, Kogia, and Balaenoptera, the cells of this nucleus are scattered in a peculiar way and similarly distributed. They first come into view in the lateral part of the spinal tract of the vestibular nerve which caps the restiform body. Next some appear along the lateral vestibular root lateral to the restiform body. Soon they become most abundant in the lateral part of the spinal tract and also in the gray matter directly dorsal to it. Medium large cells belonging to the DEITERS' nucleus are seen mostly in the dorsomedial part of the spinal tract and the adjacent gray matter. Only a few large cells are observed here. The cells of the DEITERS' nucleus are numerous in number and widely distributed transversely, but is of short duration in orocaudal direction (in Kogia only 2mm). Only a few cells of the MARTIN'S nucleus are seen in Kogia and Balaenoptera borealis in the ventral part of the spinal tract medial to the restiform body.

The tractus vestibulo-spinalis is very prominent in Lagenorhynchus and Tursio, due probably to the immature development of other fiber tracts in

the brain stem. It arises, as a collection of massive fibers, from the dorsolateral part of the DEITERS' nucleus, and arches down ventrally until it reaches the ventromedial part of the reticular formation. This tract is fairly distinct in Kogia, but in Balaenoptera it is hardly distinguishable.

Resumé

1. Structures of the acoustic system were comparatively studied in serial sections of the brain stems of six kinds of whales (Lagenorhynchus, Tursio, Kogia, Physeter, and two species of Balaenoptera).

2. In dolphins, the dorsal structures of mid-brain, pons and oblongata are remarkably shifted caudally. This phenomenon, to which the term, "telescoping" of the brain stem may be applied, is peculiar to dolphins. It is also noteworthy that the brain stem of dolphins is narrow transversely and broad dorsoventrally.

3. No "telescoping" of the brain stem was observed in Kogia, Physeter and Balaenoptera. Its form in these animals is broad transversely and narrow in dorsoventral direction, being hardly different from the brain stem of other mammals in general.

4. In Odontoceti (dolphins, Kogia, and Physeter), the ventral nucleus of the cochlear nerve, corpus trapezoides, superior olive, trapezoidal nucleus, lateral lemniscus with its nucleus, nucleus et brachium colliculi caudalis are all in a very good stage of development. They are overwhelmingly large compared with other structures in the brain stem. MONAKOW'S striae acusticae are well developed in dolphins, but less prominent in Kogia. The HELD'S tract is highly developed both in dolphins and other toothed whales. The superior olive and the lateral lemniscus of Kogia and Physeter are larger than those of dolphins, probably the largest among all mammals.

5. In Balaenoptera the ventral nucleus of the cochlear nerve and the corpus trapezoides are fairly well developed, but the superior olive is small in size and the trapezoidal nucleus, large as it is, falls short of that of Odontoceti. This is one of the remarkable differences between Odontoceti and Mystacoceti. The superior olive of Balaenoptera presents a far darker aspect, as this nucleus contains many myelinated fibers. Their lemniscus lateralis with its nucleus is somewhat retarded in its development compared with that of Odontoceti. The nucleus of inferior colliculus is fairly large

but inferior to that of Odontoceti in its dimensions. MONAKOW's striae acusticae are meagre in development and no HELD's tract was observed in Balaenoptera.

6. That there is noteworthy difference between Odontoceti and Mystacoceti in their acoustic sense is easily surmised through the study of their brain stems. The acoustic sense of Odontoceti seems to be sharp and to have reached a peculiar stage of development which is quite unique among all the mammals. The acoustic sense of Mystacoceti, though probably not inferior to that of land-living animals in acuteness, seems to be very different from that of Odontoceti. Discussions on the Mystacoceti's acoustic sense through the study of that of Odontoceti are in no way justified.

7. The corpus ponto-bulbare of ESSICK is very well developed in Kogia and in a slighter degree in Balaenoptera. In observing the end nuclei of the cochlear nerve, the existence of this gray matter should always be borne in mind.

8. Of all the vestibular nuclei only the DEITERS' nucleus is well developed in the whales we have studied. The cells of this nucleus are widely distributed in a peculiar way, a part of it lying lateral to the restiform body. The vestibulo-spinal tract is most prominent in the brains of fetal dolphins. It also seems to be well developed in Kogia and Balaenoptera, but in these animals the myelinization of fibers in the brain stem is far advanced and the vestibulo-spinal tract is not so sharply distinguished from adjacent structures.

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List of abbreviations in figs. 1—14

bc ……Brachium conjunctivum
bck ……Decussatio brachiorum conjunctivorum
brp ……Brachium pontis
cc……Crus cerebri
cgm……Corpus geniculatum mediale
ci ……Colliculus caudalis
cibr ……Brachium colliculi caudalis
coch……N. cochleae
comci …Commissura colliculi caudalis
cpb ……Corpus pontobulbare
cpbf……Fibers belonging to Corpus pontobulbare
crs ……Corpus restiforme (restiform body)
cs……Colliculus rostralis
ctr ……Corpus trapezoides
fs……Fasciculus solitarius
hl……Fasciculus longitudinalis medialis
Ht ……HELD's tract
ll ……Lemniscus lateralis
Msa……MONAKOW's striae acusticae
nci ……Nucleus colliculi caudalis
nll ……Nucleus lemnisci lateralis
np……Nuclei pontis
ntr ……trapezoidal nucleus
nv ……Nucleus ventralis n. cochleae
nvd ……pars dorsalis } Nucleus ventralis n. cochleae
nvv ……pars ventralis }
om ……Nucleus olivae accessorius medialis
osup …Superior olive (Nucleus olivaris metencephali)
osupl …pars lateralis } superior olive
osupm…pars medialis }
sgc ……Stratum griseum centrale
sn……Substantia nigra
tds ……Tractus spinalis n. vestibuli
tsves …Tractus solitarius n. vestibuli
tts ……Tractus tectospinalis
tvs ……Tractus vestibulospinalis
ves ……Redix n. vestibuli
IIIr ……Radix n. oculomotorii
IVr ……Radix n. trochlearis
Vsp……Tractus spinalis n. trigemini
Vr ……Radix n. trigemini
Vsens …Nucleus terminalis n. trigemini
VIr ……Radix n. abducentis
VIIg……Genu (internum) n. facialis
VIIn……Nucleus originis n. facialis
VIIr……Radix n. facialis
VIII……N. statoacusticus (N. vestibuli + N. cochleae)
X ……N. glossopharyngicus et n. vagus
Xr ……Radix n. vagi
XIIr……Radix n. hypoglossi

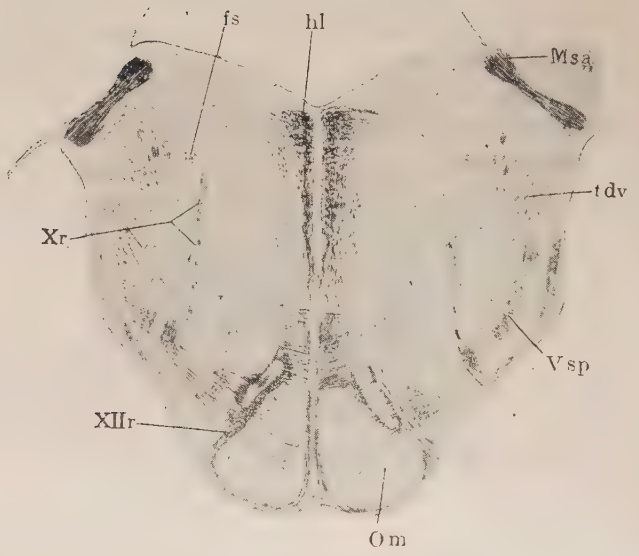

Fig. 1. Lagenorhynchus (x 3)

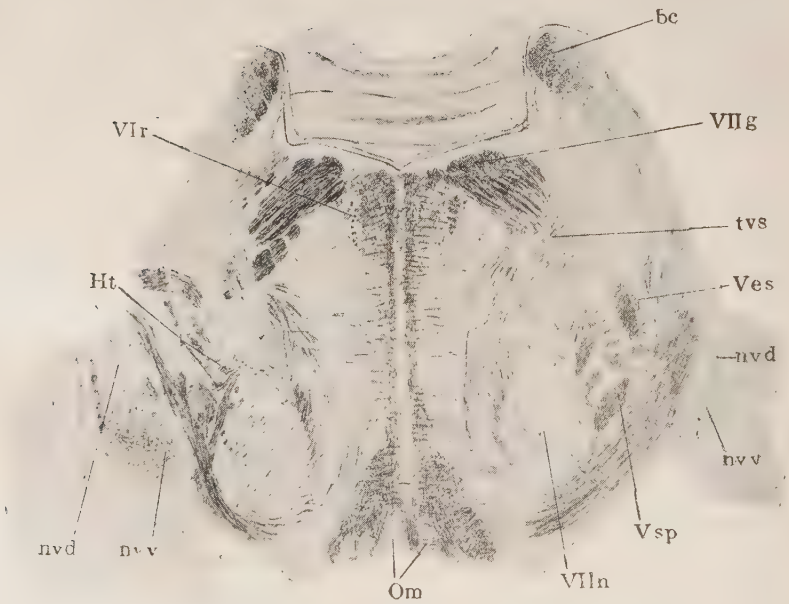

Fig. 2. Lagenorhynchus (x 3.7)

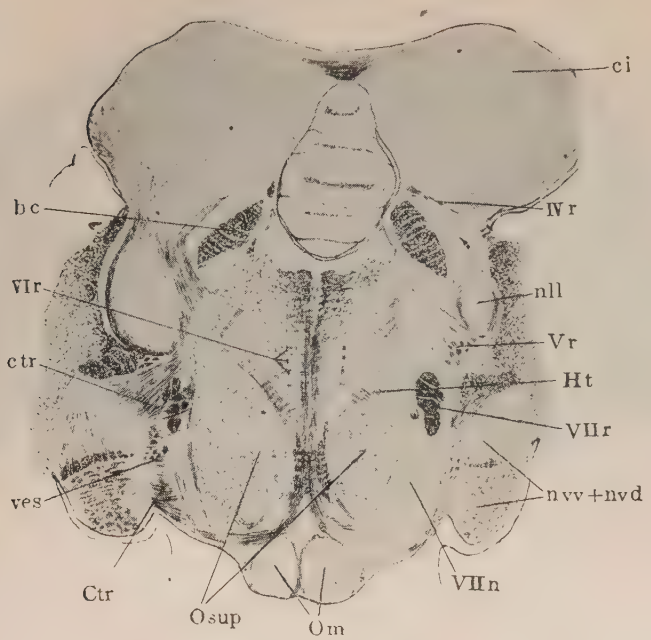

Fig. 3. Lagenorhynchus (x 2.6)

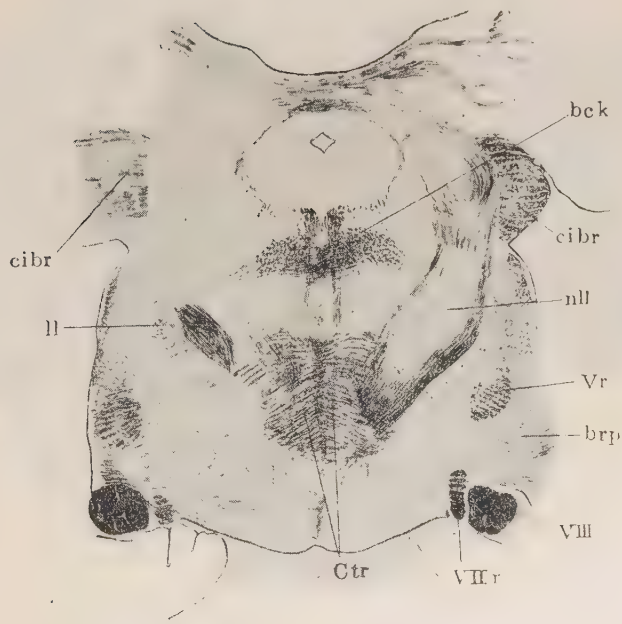

Fig. 4. Lagenorhynchus (x 2.2)

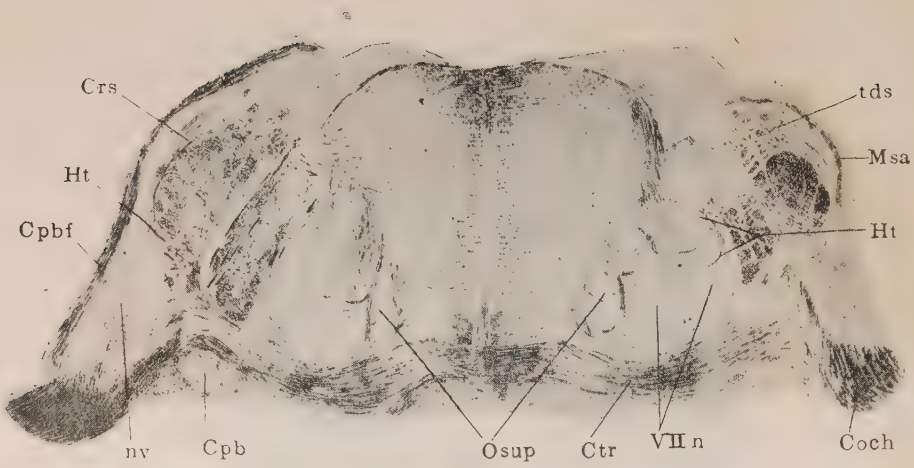

Fig. 5. Kogia (x 6.8)

Fig. 6. Kogia (x 3.5)

Fig. 7. Kogia (x 3.2)

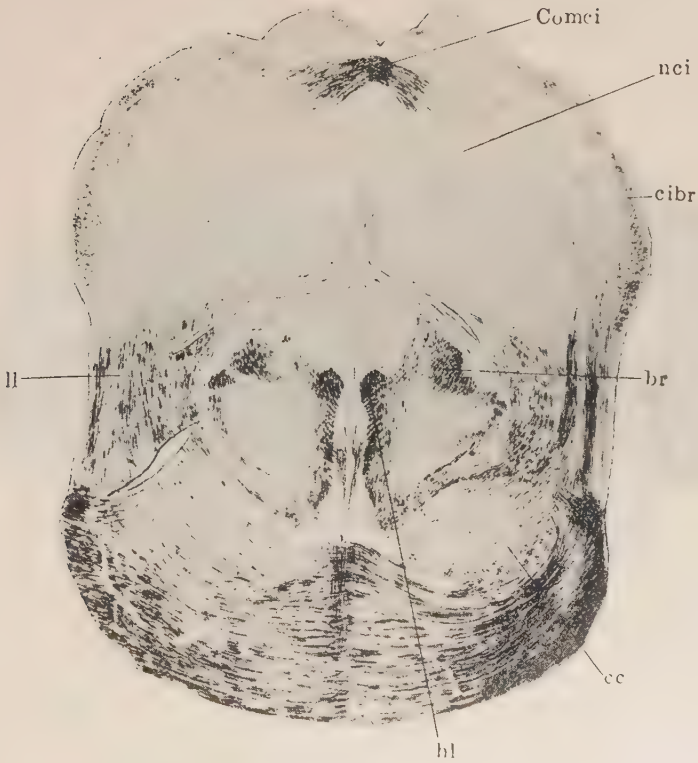

Fig. 8. Kogia (x 2.8)

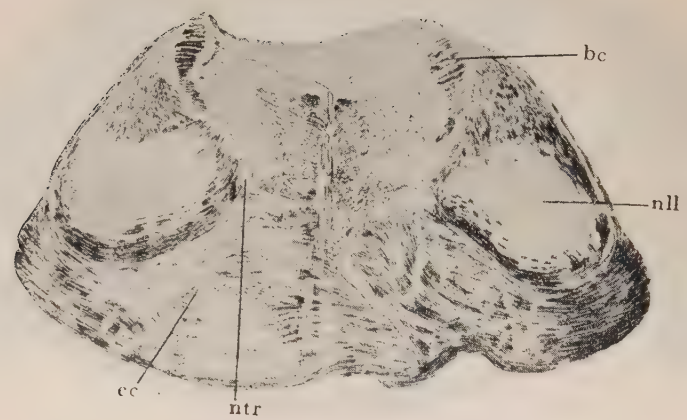

Fig. 9. Physeter (x 1.6)

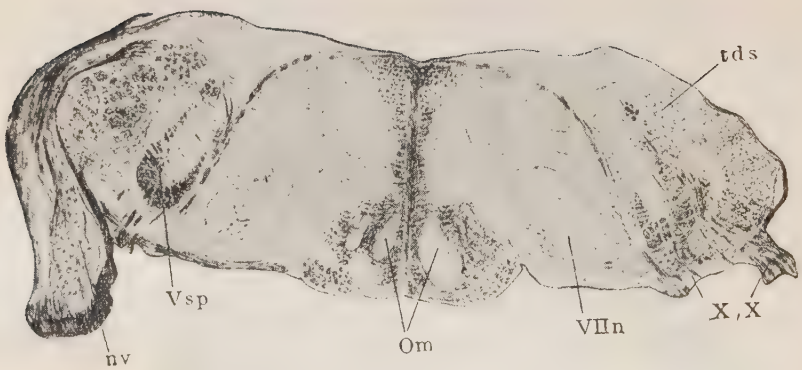

Fig. 10. Balaenoptera borealis (x 2)

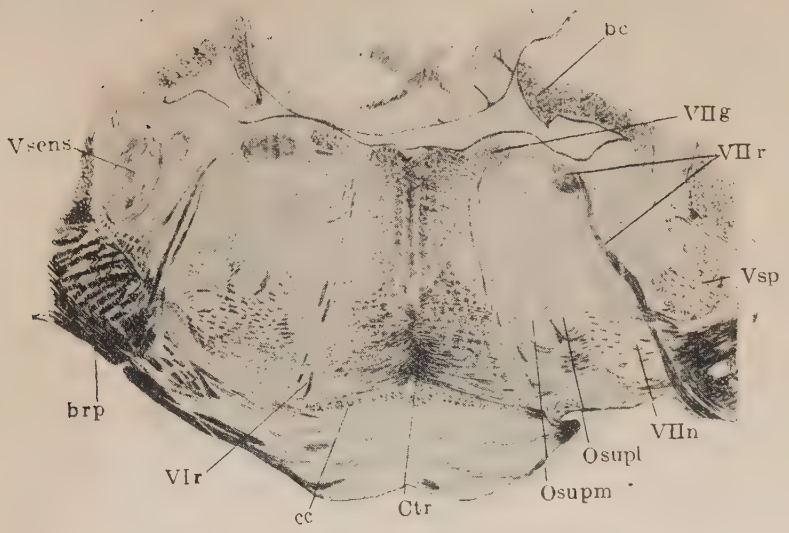

Fig. 11. Balaenoptera borealis (x 1.9)

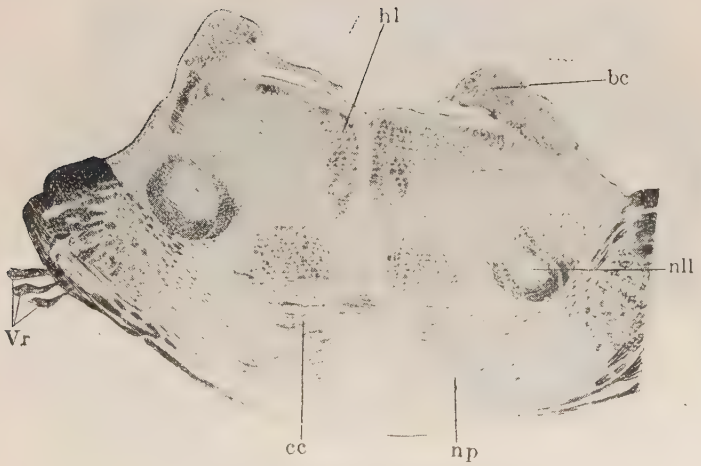

Fig. 12. Balaenoptera borealis (x 1.4)

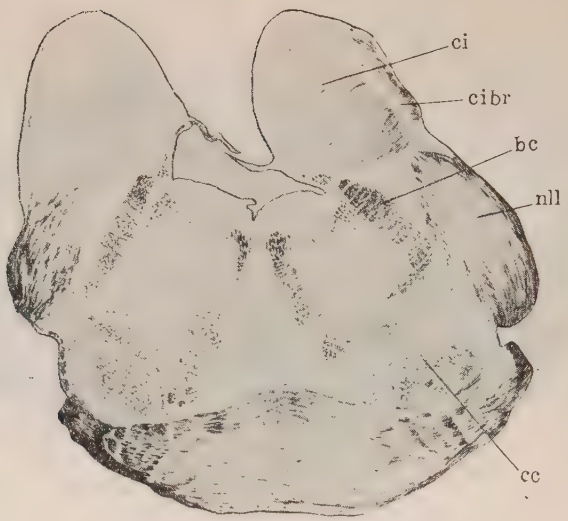

Fig. 13. Balaenoptera borealis (x 1.8)

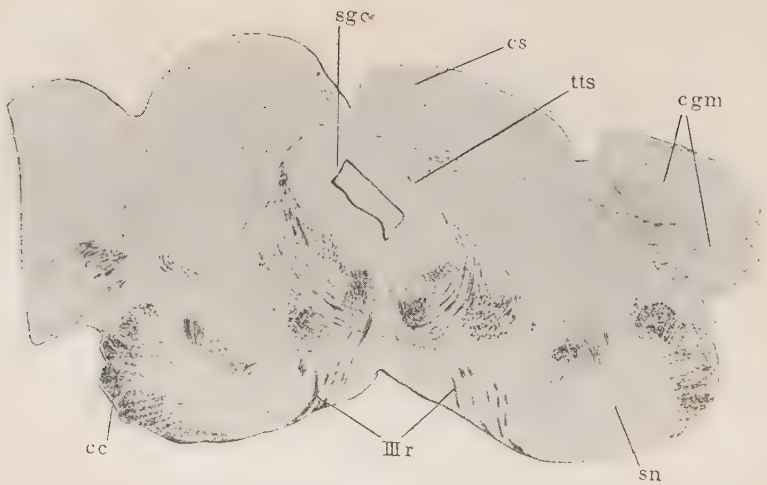

Fig. 14. Balaenoptera borealis (x 1.6)

Auditory Organ of the Whalebone Whales

(Preliminary Report)

Munesato Yamada

Department of Anatomy (Director: Prof. Teizo Ogawa), Medical School, University of Tokyo

Introduction:—

This is the first report of my studies upon the sensory organs of Cetaceans. The present investigation on the structure of Cetacean auditory organ was begun in 1946, and has made some noticeable progress especially during the Antarctic expedition on board the Japanese whaling factory ship the "Nissin Maru No. 1" of the Taiyo Fishery Company in the season of 1947—48.

Sensuous life-mode of whales is very much interesting, and important as well, not only from the biological point of view, but also for the practical side of whaling. Their sense of hearing must be very important for maintaining their lives in the water. It is commonly said among whalers

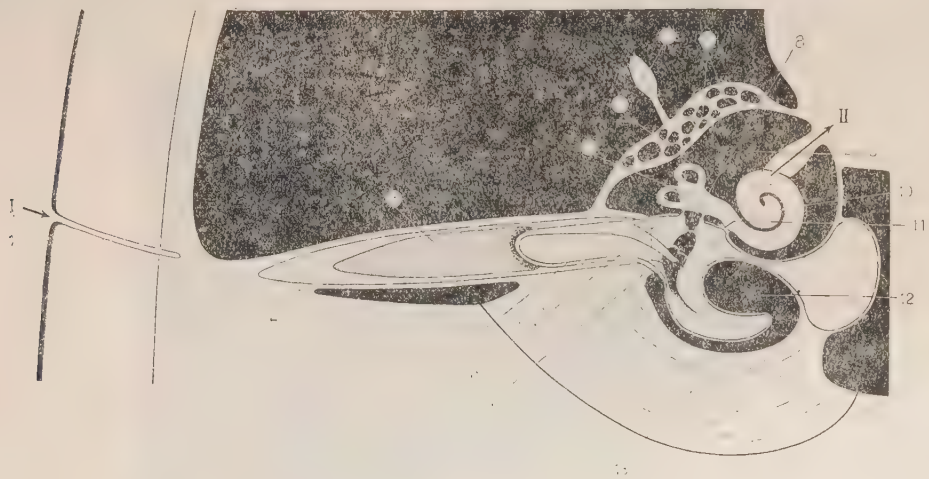

Fig. 1a. Diagram of the auditory organ of *Balaenoptera* in frontal section. ↑I. shows outer ear hole, ↑II. Inner acoustic meatus. 1. Lateral portion and 2. Medial portion of the outer acoustic meatus, 3. Plug of ear-wax, 4. Vestibule, 5. Semicircular canal, 6. Ligamentous cord, 7. Sesamoid ossicles, 8. Stapes, 9. Periotic bone, 10. Cochlear labyrinth, 11. Cochlear fenster, 12. Tympanic bone, 13. Thick fibrous layer of connective tissue, 14. Triangular ligament between the tympanic membrane and malleus, 15. Thin pedicles which fuse the tympanic and periotic bones, 16. Tympanic membrane, 17. Incus.

that whales are very sensitive to the sounds in the water. We can give some examples to make this view easily be accepted, although in this place they are not touched at all.

Materials :—

Materials here treated are confined to two species of the genus *Balaenoptera*, i. e., *Balaenoptera musculus* and *Balaenoptera physalus*, because other species could never be caught by the Japanese in the Antarctic. Being blessed with abundant materials, both adult and foetus, I made observations about the organ macroscopically on board, and brought home some materials for further studies.

Here, merely an outline of my observations will be reported, mainly about the fin whale (*B. physalus*), and further details will be published in the near future together with histological studies, considering at the same time some other members of Cetacea.

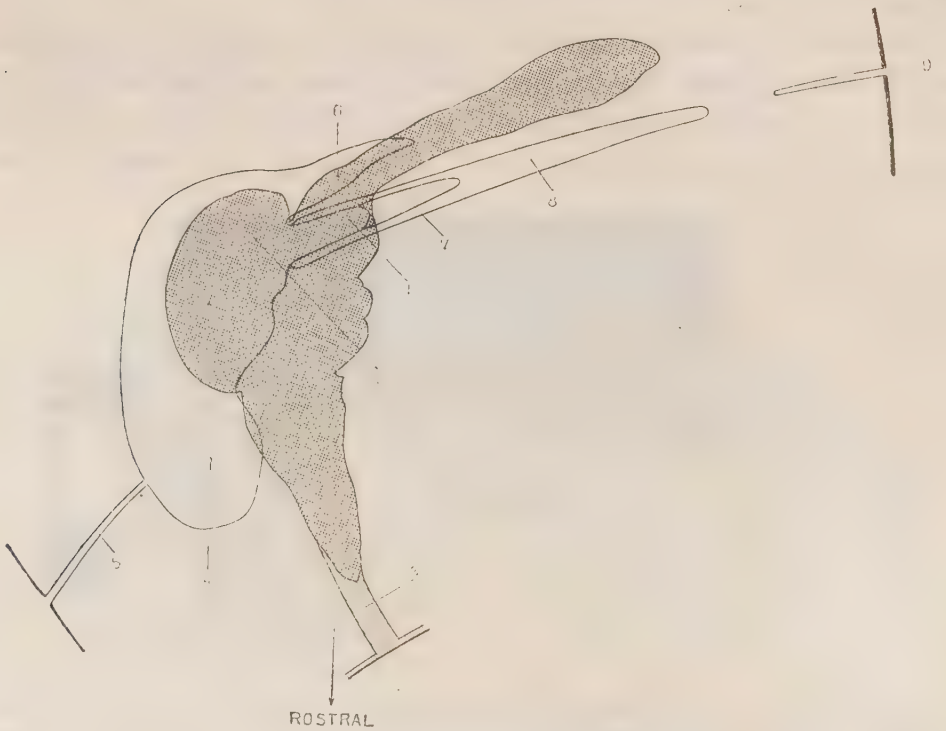

Fig. 1b. Diagram of the auditory organ of *Balaenoptera* in horizontal section, illustrating the topographic relation of the tympanic cavity and its adjoining expansions to the petrotympanic bone, which is shown half-toned in the background. 1. Tympanic membrane, 2. Petrotympanic bone, 3. Rod of connective tissue straining the pro-otic leg to the periost outside, 4. Pterygoid fossa, 5. Eustachian tube, 6. Blind diverticle of the tympanic cavity, 7. Plug of ear-wax, 8. Medial portion, and 9. Lateral portion of the outer acoustic meatus.

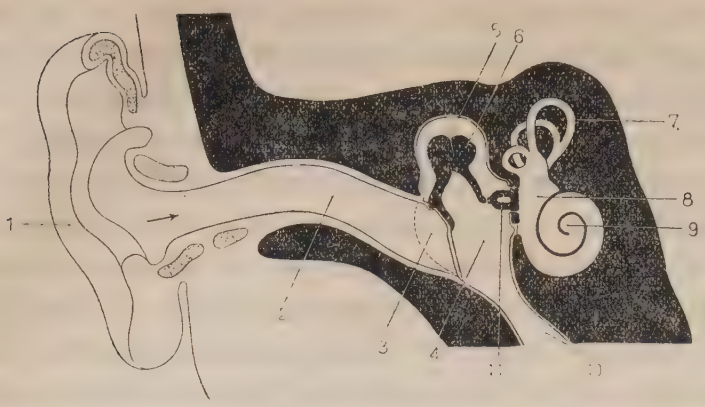

Fig. 1c. Diagram of the auditory organ of the human being in frontal section. 1. Auricle, 2. Outer acoustic meatus, 3. Tympanic membrane, 4. Tympanic cavity, 5. Malleus, 6. Incus, 7. Semicircular canal, 8. Vestibule, 9. Cochlear labyrinth, 10. Eustachian tube, 11. Stapes.

Fig. 1a is a diagram showing the structure of the auditory organ of *Balaenoptera* in frontal section, to be compared with another diagram (Fig. 1c) of the human ear.

Outer Ear:—

The outer ear hole of fin whale is small, opening at the bottom of a longitudinal short groove, which runs parallel to the ventral grooves, and lies about halfway from the eye to the anterior edge of the fore limb (Fig. 2). It is usually a horizontal slit, and admits the little-finger no further

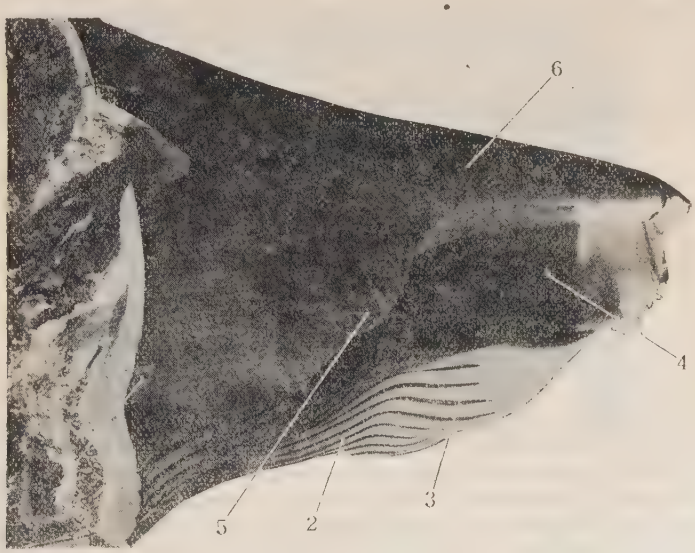

Fig. 2. Head of a fin whale showing the outer ear hole (1) and its topographical relation to the eye (5). 1. Outer ear hole, 2. Ventral grooves, 3. Mandible, 4. Baleen, 5. Eye, 6. Maxilla.

than the third phalangeal joint, measuring about half an inch in diameter.

The outer acoustic meatus is obviously degraded, devided into two portions, the lateral and the medial. Of these two portions, the lateral is far more rudimental and far shorter in length than the medial. The lateral portion is buried in thin blubber, and usually torn off with the blubber at the time of dissection. On the contrary, the medial portion is pretty well preserved in the acoustic furrow, which lies on the lower side of the squamosal bone. In my former observation of a female fin whale at Akkeshi, Hokkaido (1947), the lengths of the two portions measured 13 cm and 45 cm respectively, and to the consequence, the outer acoustic meatus was broken for the interval of about 30 cm. This fact of discontinuance of the acoustic meatus in the whalebone whales, however, has strange enough been missed by some authors worked on this same subject (Lillie, 1910; Hinoura, 1938). And so far as learned by me, Remington Kellogg (1928) alone has presented an accurate description of this fact about the humpback whale (*Megaptera*).

The tympanic membrane is rather thick, and projects outwards about 6 cm into the acoustic meatus in a shape of a finger-sac, slightly flattened in vertical direction; it is connected with the rigid malleus by a ligamentous cord (Fig. 1a and 3). Thus, the membrane obviously lost its original function as a drum, and its outer surface is coated tightly with a plug of ear-wax. With this tympanic membrane, the outer ear adjoins the middle ear.

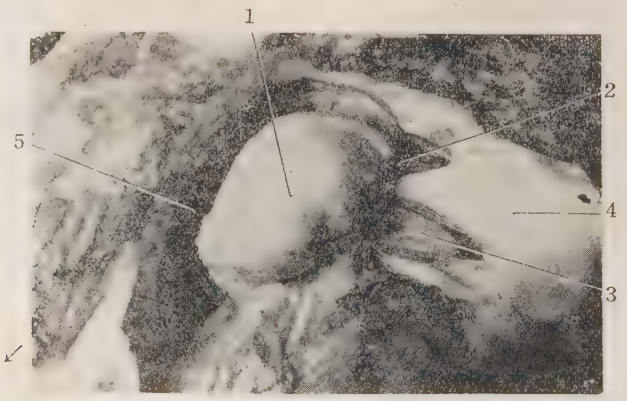

Fig. 3. Photograph showing the tympanic bone and part of the tympanic cavity, together with the tympanic membrane. Ventral view, right side. After removal of the thick fibrous layer partially (4). Arrow shows the rostral direction. 1. Tympanic bone, 2. Blind diverticle of the tympanic cavity, 3. Tympanic membrane, 4. Fibrous layer covering the auditory region from the lower side, 5. Tympanic cavity.

Middle Ear:—

The most conspicuous character of the middle ear of the whalebone whales is the morphology of the tympanic bone, which is also known by the name of "tympanic bulla," and the relation of this bone to the periotic (os perioticum s. petrosum). These two bones are fused together with two thin but broad pedicles as is shown in Figs. 6 and 7. The two bones are, altogether, sometimes called as the petrotympanic (os petrotympanicum) or tympano-periotic. The tympanic membrane is situated in the bony tunnel between these two bones, between these pedicles.

The tympanic bone is very hard like quartz and compact like ivory, and is shaped like a cowrie shell, with a deep depression on its upper surface and lies so as its longitudinal axis to be parallel to that of the whale body. The middle ear cannot easily be seen at the dissection because a particular white, thick and hard layer of connective tissue covers this region from the lower side (Figs. 1 and 3). But, in the region of the tympanic alone, no tight connection exists between this bone and the fibrous layer but a little amount of adipose tissue. The tympanic bone is, therefore, comparatively free from the fibrous layer, and consequently, the heavy tympanic bone is often dislocated by the external force or through inertia when this layer is taken off, for the pedicles are thin and easily broken.

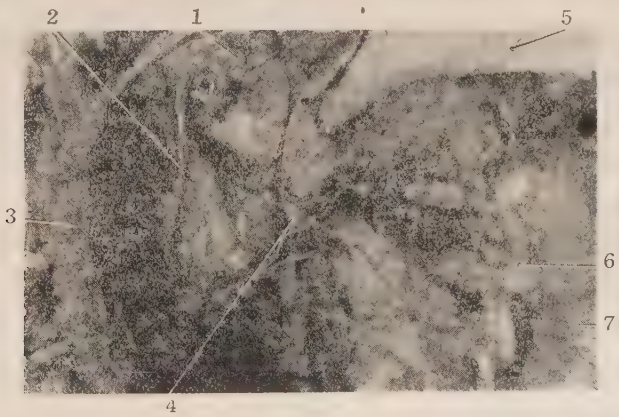

Fig. 4. Photograph of the tympanic cavity in full view (right side, same specimen with Fig. 3). Drawing-out line 4 shows the rostral direction. Photo shows the mucous folds and vesicles on the spherical portion of the periotic bone. Incus is seen in the center, joining with stapes, but the joint between incus and malleus is broken, the tympanic bone being overturned to the right. 1. Vesicular formation, 2. Mucous fold, 3. Medial wall of the tympanic cavity, 4. Incus, 5. Connective tissue, 6. Malleus, 7. Tympanic bone.

Tympanic Cavity :—

The tympanic cavity is, in whalebone whales, a room that lies between the tympanic and the periotic bones, situated likewise to the land animals next to the tympanic membrane (Figs. 1a., 4 and 7). The periotic makes the ceiling of the tympanic cavity with its spherical portion, in which there lies the inner ear, viz. cochlea, vestibule and semicircular canals.

The tympanic cavity has a remarkable expansion in the depression of the tympanic bone ; it is also expanded rostrad into the pterygoid fossa and caudo-laterad into a pointed blind diverticle in the above mentioned thick layer of connective tissue (Fig. 1b, 3 and 6). The cavity has also a finger-like expansion in the tympanic membrane , thus, four expansions are attached to the tympanic cavity, of which the pterygoid fossa is the largest (Fig. 1b).

The mucous membrane which covers the tympanic cavity and its expansions is rugged with various folds and vesicular formations. These formations are most complex on the ceiling of the tympanic cavity, that is, the spherical portion of the periotic bone (Fig. 4). The pterygoid fossa is to be considered as an expanded posterior portion of the Eustachian tube. The Eustachian tube begins on each side at the upper lateral wall of the nasal passage instead of the pharyngeal cavity, and runs about 30 cm backwards to somewhat lateral direction to the pterygoid fossa, and joins it at its ventral floor (Fig. 1b).

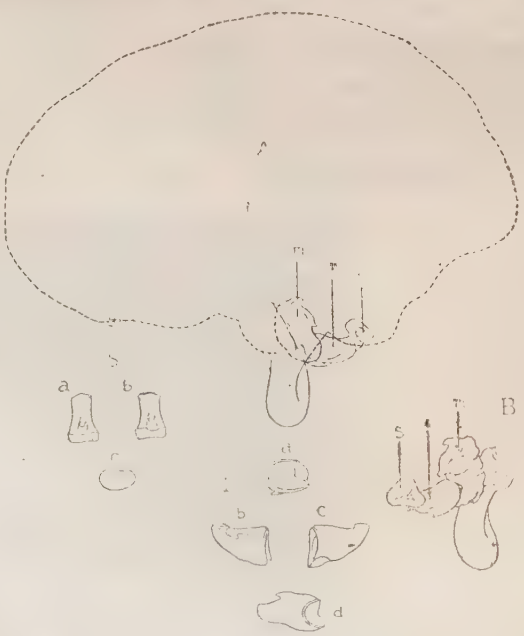

Fig. 5. Tympanic ossicles. Right side. The dotted line shows the contour of the tympanic bone A. (ventral view), and B. (dorsal view) in toto. m. Malleus, i. Incus, s. Stapes. I. Incus seen from : a. Rostro-ventral, b. Lateral, c. Mesial, d. Ventro-caudal. S. Stapes seen from : a. Caudo-mesial, b. Rostro-lateral, c. Basis stapedis.

Tympanic Ossicles :—

Like other mammals, across the tympanic cavity, there lies a chain of the tympanic ossicles : malleus-incus-stapes. Forms of these ossicles are different to some extent from those of the

land mammals. The malleus, above all, is fused to the tympanic bone, and is so located as to coincide with the phonetic focus for the depression of the tympanic bone, which I believe, to function as a resonance box, an amplifier of sounds in the water.

Thus, three ossicles make a continuously articulated bony chain, until finally the stapes inserts its bottom into the vestibular fenster. The connection between stapes and vestibular fenster of the inner ear is not ankylosed, and accordingly, the vibrations of the stapes are considered to be transmitted into the cochlear lymph through the fenster. The tympanic ossicles are shown in Fig. 5.

The chorda tympani of the facial nerve was not observed in the tympanic cavity.

Inner Ear:—

The inner ear, namely vestibule, cochlear labyrinth and semicircular canals, together with inner acoustic meatus containing the auditory and the

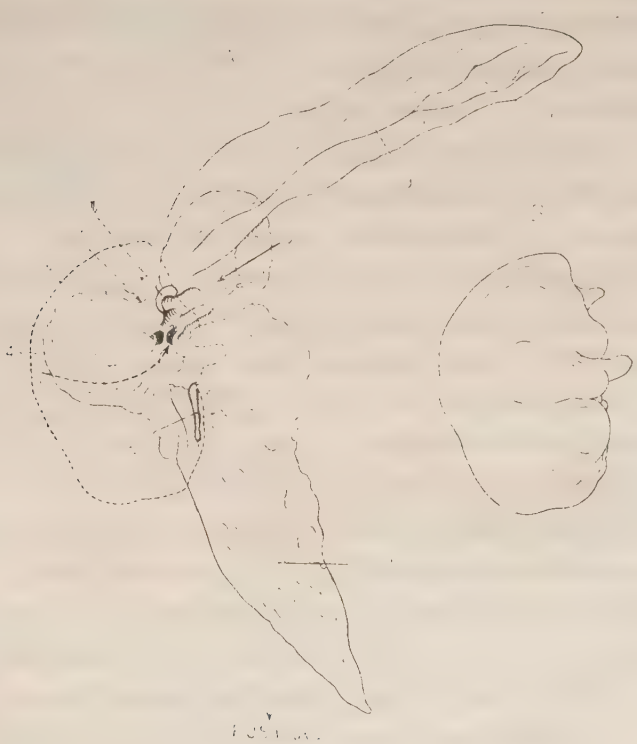

Fig. 6. Petrotympanic bone. Right side, ventral view. The tympanic bone is dislocated to the right (B), to illustrate the labyrinthic portion of the periotic (A). 1. Caudal pedicle, 2. Cochlear fenster, 3. Vestibular fenster, 4. Labyrinthic portion, 5. Facial nerve canal, 6. Rostral pedicle, 7. Pro-otic leg, 8. Furrow for the outer acoustic meatus, 9. Opisthotic leg.

facial nerves, and the Falloppian duct of endolymph, etc., lie in the central spheric portion of the periotic bone. For this reason, the spheric portion is also called as the labyrinthic. The vestibular fenster is situated in a depression on the under side of this dome-shaped labyrinthic portion at its lateral border. The cochlear fenster lies on the posterior surface of the labyrinthic portion, a little distant behind the vestibular fenster (Fig. 6). The cochlear labyrinth measured, in one case of fin whale (77 feet female), 19 mm in diameter, the modiolus being 10 mm in length. The rotation of the cochlear canal is about two and a half as in the human ear. The cochlea exceeds that of the mankind more than twice in each dimension, while the three semicircular canals are not larger than the human case. The bony tissue of the labyrinthic portion is also as hard and as compact as the tympanic bone, but the thin layer that faces to the cochlea is not so compact as its surrounding tissue. Such being the structure, the cochlea is easily broken during chiseling even with the greatest care. But, the medial part of this portion, which is perforated by the auditory nerve, the Falloppian duct and the facial nerve, and which takes part in the formation of the basis of the cranial cavity is as loose as other bones such as vertebrae.

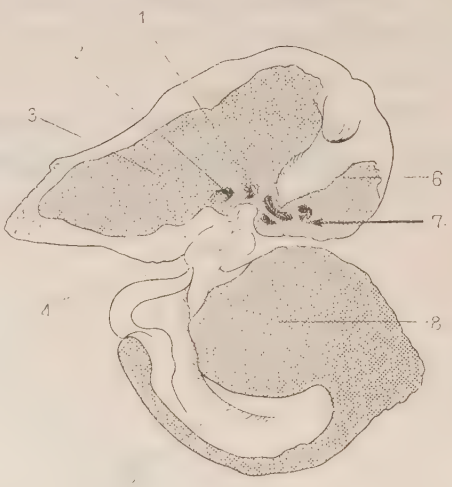

Fig. 7. Petrotympanic bone. Right side, frontal section. 1. Vestibular fenster, 2. Opening of facial nerve canal, 3. Periotic bone, 4. Malleus, 5. Depression of the tympanic bone, 6. Inner acoustic meatsus, 7. Cochlea, 8. Tympanic bone.

The periotic bone has two more portions besides the labyrinthic: the anterior pro-otic and the posterior opisthotic (Figs. 1b and 6). The former is pointed and of pyramid shape, being also hard and compact, and projects into a canal in the squamosal above the roof of the pterygoid fossa, but is coated and connected with a thick sheath of fibrous tissue. These two, making together a rod-shaped process, are connected with the periost of the squamosal through the canal above mentioned to the lateral outside of the squamosal (Fig. 1b). On the contrary, the opisthotic portion lies in the skull firmly between the squamosal and the exooccipital bones. In other words,

the three portions may be said that the two of them, the pro-otic and the opisthotic portions form a right angle at the site where the labyrinthic portion lies.

The opisthotic portion is thin and compact at its root, but broadens and extends towards its extremity, and at the same time it diminishes in compactness. These two extremity portions, I would hereafter call as "Legs" (cruri).

How the Organ Functions :—

The problem how the Cetacean auditory organ functions, has been discussed for a recent century. Although many authors such as Buchanan, Claudius (1858), Denker (1902), Boenninghaus (1903), Lillie (1910), Abel (1912), Kellogg (1928) and Hinoura (1938) have set up some theories for this problem, none of them has been hitherto regarded to be perfect and indisputable. The reason why this problem has not been settled is that, the two suborders of Cetacea, toothed and baleen whales are often treated equally, in spite of the great difference in structure between them, and that perhaps for the difficulty of dissection, some structures having been missed by some or all authors.

I myself would lay special weight on the structural relations of the above described cruri to the skull and the peculiar ankylose of the tympanic bone to the periotic, and explain the auditory function of the whalebone whales as follows :— In short, the entire petrotympanic bone is set into vibration by the sounds, transmitted through the surface of the body, especially from the head, at that time the opisthotic leg functions as fulcurum and propagating apparatus (Abel), while the pro-otic leg strains the entire organ to the periost outside the skull, and thus, making the vibration more effective, limits vibrations of the petrotympanic to a certain extent. And to make my suggestion more certain, I will mention some other structures which seem to have escaped the notice of previous researchers. Between the upper side of the labyrinthic portion and the under side of the skull, there exist in the fibrous layer, scores of sesamoid ossicles, large and small in size, which seemingly function as buffers and are considered to make the whole organ noiseless. Besides, from this fibrous tissue, a thin but strong cord extends upwards into the squamosal bone of the skull and ends in a swelling (Fig. 1a). Sesamoid ossicles are also observed at the entrance of the pro-otic canal in the roof of the pterygoid fossa, between

the pro-otic leg and the squamosal bone.

When the petrotympanic bone is put into vibration, it must be inertially amplified by the tympanic with its heaviest mass through its peculiar attaching mode to the periotic. Of the two flattened thin pedicles, which fuse the tympanic to the labyrinthic portion, the posterior one is situated at right angle to the anterior one (Fig. 6), in order to maintain the heavy mass of the lateral lip of the tympanic at the time of vibration (Fig. 7).

These facts seem to be essential for the functioning of the tympanic as the amplifier of the vibration and as the resonance box.

Moreover, the depression of the tympanic bulla and other expansions of the tympanic cavity may also well be considered to be resonators, and put the malleus into utmost vibration. In both cases, the vibration can be transmitted through the bony chain by the basis of stapes directly into the cochlea. As described before, the ventral surface of the tympanic bone is relatively free from the fibrous layer which covers the entire auditory region from the lower side, This relation makes the tympanic vibrate freely and thus, make my vibration theory in the ear of whalebone whales more reasonable. This fibrous layer, in my opinion, protects the auditory organ from tremendous pressure of sea water, when whales dive down to a great depth.

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Chemical studies on the Freshnes of whale Meat III

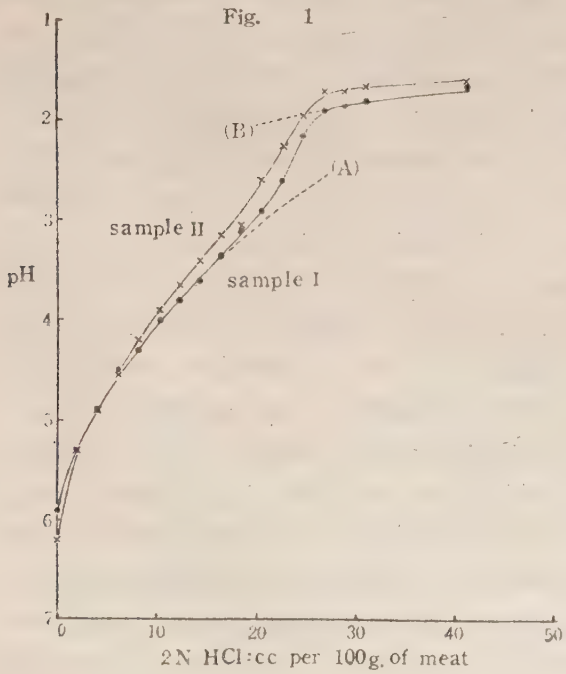
Effect of Hydrogen-Ion Concentration on Decrease in Freshness and Titration Curve of Whale Meat with HCl and Na_2CO_3

Tadashi Nakai

In order to find out the effect of pH on decrease in freshness of whale meat, buffer solution of pH 2; 4.2; 5.9 and 7.7 was added to minced whale meat and left standing for 144 hours at room temperature and the changes in the quantity of volatile basic nitrogen and formol titrating nitrogen were studied. At pH 2 and 4.2, it was observed that formol titrating nitrogen increased slightly but volatile basic nitrogen showed almost no increase. At pH 5.9 and 7.7, both nitrogens showed a very large increase. That is, decrease in freshness may be stopped at pH lower than 4.2, pH 4.2 is very nearly the same as the growth limit pH of bacteria on the acidic side.

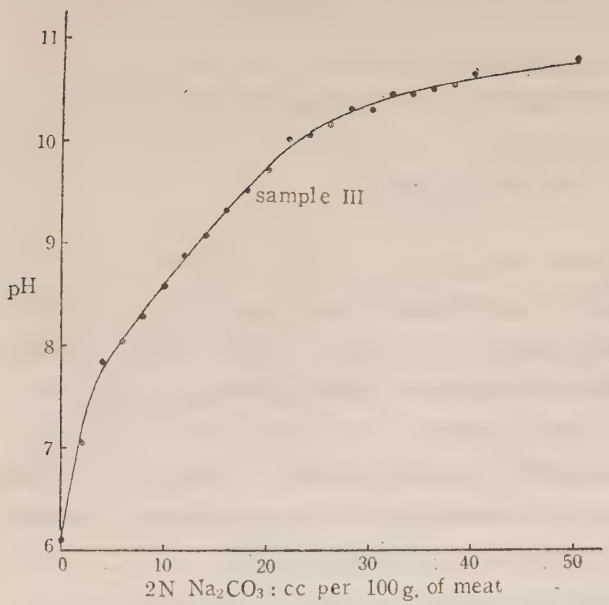
From this result, the author planned a storage experiment on whale meat at pH outside the growth range of bacteria. However, Nakae[1] reports that, with the object of industrial treatment of sardine, he was successful in preventing spoilage of sardine by storage at pH below 3.2 by using HCl. HCl was used in the author's experiment and first of all, in order to find out the required quantity of HCl, 2*N* HCl was used and titration curve of whale meat was obtained (Fig. 1). At the same time, titration curve by 2*N* Na_2CO_3 was obtained because it was necessary for other experiment (Fig. 2). Samples in all cases were baleen whale meat.

Fig. 1



Nakae[2] obtained titration curve with HCl on many kinds of fish and reports that generally there is less buffer action

Fig. 2



between pH 6—4 and appears strongly between the pH 4—3 range. According to the result obtained by the author, with whale meat, buffer action is weak between pH 2—3 and pH 5—7.7 range and appeared relatively strongly at other parts. As can be observed from Fig. 1, buffer capacity was somewhat small until up pH of about 3, in Sample II which is high in fat content and low in other constituents when compared with Sample I which has the opposite content of the various constituents.

Titration curve with HCl clearly indicates that it is composed of two types of curves. For example, in the curve of Sample I there is a curve having an extension (A) and a curve with extension (B) and it shows that there is a combination of both curve in between. That is, it is assumed that curve (A) and curve (B) each indicates a separate reaction. Therefore, according to the graph, a reaction shown by curve (A) only takes place up to pH of about 3.5, next, the reaction shown by curve (B) begins and gradually reaction of curve (A) diminishes and reaction of curve (B) only takes place at pH lower than 2. The same can be said for Sample II. Thus, it is very interesting to note that clearly, two reactions takes place one after the other so it might be able to clarify a part of the chemistry of decrease in freshness by investigating this further.

If pH 3 is assumed to be the objective pH for storage, from the above results, about 200 c.c. of 2*N* HCl will be required per 1 kg. of whale meat. Thus, when a lump of whale meat is soaked in half its quantity of water which also contains the aforementioned percentage of HCl, removed after 5 days and boiled together with water, decomposition of the fleshy part was great and also, the meat had an acidic taste. Therefore, this method

can be applied for studying the quantity of volatile basic nitrogen only because actually it is accompanied by the aforementioned changes so it is inappropriate to apply this in practical cases.

Gratitude is expressed to Dr. Tsutomu Maruyama for his kind instructions and review, and appreciation is expressed to Mr. Ryusuke Fukazawa for his assistance in the expriment.

Experiment

Changes in volatile basic nitrogen and formol titrating nitrogen in whale meat at time of adding buffer solution.

To 2 g. each of minced baleen whale meat 18 c.c. of buffer solution of pH 2, 4.2 (mixed solution of HCl—NaAc); 5.9, 7.7 (mixed solution of phosphate) was added and after standing for 144 hours at room temperature in summer, 30 c.c. of absolute alcoholic $N/100$ H_2SO_4 was added to stop further changes and the following analysis made.

Volatile basic nitrogen— The above mixture is made slightly acidic, boiled mildly on a water bath to evaporate the alcohol and made to about 20 c.c., 1 g. of MgO added and as previously reported[1], volatile basic substance was collected into $N/50$ H_2SO_4 by the aeration method and titrated back with $N/50$ NaOH.

Formol titrating nitrogen— The above mixture is made slightly acidic, heated in a mildly boiling water bath for 15 minutes, drained off and washed 3 times by heating for 5 minutes in water bath with 10 c.c. of distilled water each time. 1 c.c. of 20% sulfosalicylic acid is added to the filtrate and washing and left standing for one night. It is then filtered, washed, filtrate and washing made to 40 c.c. and 25 c.c. of this is titrated by the usual method.

The result obtained was as follows:

	Volatile basic Nitrogen (mg/100 g.)	Formol titrating Nitrogen (mg/100 g.)
At start	43.4	100
pH 2	41.0	131
pH 4.2	44.1	154
pH 5.9	361	555
pH 7.7	More than 389	969

Titration curve.

The sample whale meat were three kinds of baleen whale meat (frozen) and their composition were as follows:

	Moisture %	Crude Protein %	Crude Fat %	Ash %
Sample I	72.65	21.48	3.97	1.02
Sample II	63.80	19.57	15.13	0.85
Sample III	76.89	20.70	1.65	0.95

a) Titration curve by HCl.

Samples I and II were used. These were minced with a meat grinder, variable quantity of 2*N* HCl and distilled water added to 10 g. of minced meat and the total quantitiy made in to 20c.c. of slushy substance. It is left standing for one night and pH determined with Yokogawa type antimony electrode hydrogen ion meter. However, pH of the sample with 0 c.c. HCl added was determined one hour after addition of distilled water.

The following result was obtained. From this, the curve in Fig. 1 was obtained.

2*N* HCl (c.c. per 100g. of meat)	0	2.05	4.11	6.16	8.22	10.27	12.32	14.38	16.45	18.49
pH Sample I	5.9	5.3	4.9	4.5	4.3	4.0	3.8	3.6	3.35	3.1
pH Sample II	6.2	5.3	4.9	4.55	4.2	3.9	3.65	3.4	3.15	3.05

2*N* HCl (c.c. per 100g. of meat)	20.54	22.59	24.65	26.70	28.76	30.81	41.08
pH Sample I	2.9	2.6	2.15	1.9	1.85	1.8	1.65
pH Sample II	2.6	2.25	1.95	1.7	1.7	1.65	1.60

b) Titration curve by Na_2CO_3

Sample III was used, treated in the same manner as a) and the following result was obtained. From this, the curve in Fig. 2 was obtained.

2*N* Na_2CO_3 (c.c. per 100g. of meat)	0	2.0	4.0	6.0	8.0	10	12	14	16	18	20
pH (Sample III)	6.1	7.05	7.85	8.05	8.3	8.6	8.9	9.1	9.35	9.55	9.75

2*N* Na_2CO_3 (c.c. per 100g. of meat)	22	24	26	28	30	32	34	36	38	40	50
pH (Sample III)	10.05	10.1	10.2	10.35	10.35	10.5	10.5	10.55	10.6	10.7	10.85

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2) Nakae: Ibid, **21**, 661 (1943).

3) This periodical No. 1, page 21.

Analytical Distillation of Vitamin A in the Whale Liver Oil

Seiichi Ishikawa, Yoshimori Omote, Yoshitaka Soma.

1. Introduction.

The presence of ordinary vitamin A and a vitamin A possessing different characteristics has been confirmed through research by Willstaedt and Jensen[1] and Nakamiya, et al[2] but recently, it has been reported by Baxter, et al[3] that kitol, a kind of provitamin A has been isolated from the unsaponifiable matter in whale liver oil, which has twice the molecular weight of vitamin A and which can be decomposed by heat into vitamin A. The authors carried on an analysis of vitamin A in the unsaponifiable matter of the Antartic whale liver oil (it is not certain whether the whale was a fin or blue whale but it is certain that it was from one of the two) by molecular distillation.

2. Analytical Distillation of Unsaponifiable Matter in Tunny Liver Oil.

First, in order to obtain an analytical distillation curve of a standard vitamin A, unsaponifiable matter of tunny liver oil, which is rich in ordinary vitamin A was used, to which was added mixed glycerides obtained by synthesis, as controlled yield oil[4] (abbreviated to C. Y. O.) and the result indicated in Table 1. was obtained. Graphically, it is as shown in Fig. 1. According to this graph, elimination maximum (abbreviated to E. M.) appears in the vicinity of 185°C and it is assumed that this is ordinary vitamin A.

Experiment 1. Synthesis of C. Y. O.

Coconut oil fatty acid is supplemented with acetic acid, butyric acid, and capric acid and equi-molar ratio mixture of fatty acid of the various carbon atom numbers were prepared, glycerine added and esterified in the presence of decalin. The mixed glycerides thus obtained were distilled in a cyclic still and divided into various fractions through a range of 130—280°C. Next, equal quantity of the various fractions were mixid and used as C. Y. O. and residue above 280°C used as R. O. (residue oil). Temperature determination was made in the middle of the heating oil bath within the evaporating cylinder. During the process, the bath was well stirred so it is assumed that it is approximately the temperature of the evaporating sur-

face. Also, the temperature of the residue oil which flowed down the heating cylinder and dropped from the bottom was also determined, from which it was discovered that the value was approximately 60°C lower than that of the oil bath. Vacuum was $10^{-2}-10^{-3}$mm. and the distance between the evaporating surface and condensing surface was 0.5 cm. Temperature was raised every 10°C and at each range, distillate for each cycle was removed. These conditions were used for all analytical distillation explained hereinafter.

Experiment 2. Analytical Distillation of Unsaponifiable Matter in Tunny Liver Oil.

Analytical distillation was carried on with 76 g. of oil of 102 liver oil unit, obtained by adding 35 g. of C. Y. O. and 35 g. of R. O. to unsaponifiable matter from 25 g. of tunny liver oil for injection purpose (400 liver

Table 1. Analytical Distillation of Unsaponifiable Matter in Tunny Liver Oil

No.	Temp. (°C)	Distillate (g)	Liver Oil Unit	Liver Oil Unit × Weight	% of Recovered Vitamin A
1	130	2.4	103	248	3.2
2	140	2.1	109	233	3.0
3	150	2.5	310	788	10.2
4	160	1.8	400	729	9.4
5	170	1.8	585	1060	13.7
6	180	2.8	598	1643	21.2
7	190	2.0	462	903	11.2
8	200	2.5	341	852	11.0
9	210	2.6	202	533	6.4
10	220	1.9	143	270	3.5
11	230	1.2	130	156	2.0
12	240	1.2	65	77	1.0
13	250	1.2	45	52	0.7
14	260	1.1	64	93	0.9
15	270	1.7	55	91	1.2

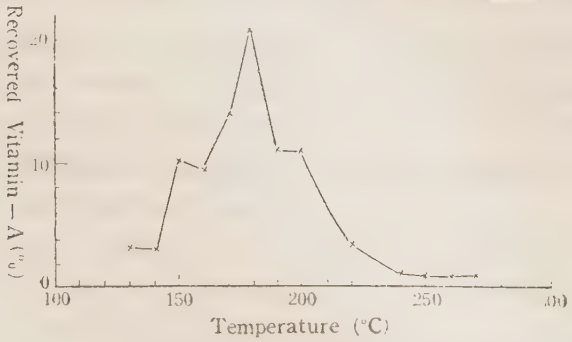


Fig. 1. Analytical Distillation Curve of Unsaponifiable Matter in Tunny Liver Oil.

oil unit). Oil distilling over at each 10°C (1 cycle) was taken, its weight and liver oil unit determined and the percentage of recovered vitamin A was calculated by the following formula:

% of recovered vitamin A=[(liver oil unit×weight of each distillate)/(liver oil unit×weight of oil before distillation)]×100%.

Shown graphically, the experimental result is as indicated in Fig. 1.

3. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil.

Analytical distillation was carried on with unsaponifiable matter of Antarctic whale liver oil under the entirely similar conditions as in the case of tunny liver oil explained above. The result is as indicated in Fig. 2. As can be clearly seen from the graph, E. M. of No. 1. is in the vicinity of 180°C and entirely coincides with the E. M. as in the case of tunny liver oil. That is, there is no mistake that this is vitamin A. Next, in the case of whale liver oil, there is a tendency of a second E. M. to appear, but in the case of Fig. 2., since the same condition as in Fig. 1. was followed strictly, the distillate of C. Y. O. above 280°C was small and the distillation was stopped here. Thus, in order to obtain the second E. M., C. Y. O. and R. O. above 280°C was newly added and distilled. As a result of this, the second E. M., as can be clearly seen in Fig. 3., appeared. The first E. M. was in the vicinity of 185°C, the same as in the previous case, so that there is no question, but the shape of the second E. M. is somewhat vague, a shape which is formed by several peaks of single substance overlapping and its temperature ranges from 270—290°C. According to Hickman[9] natural vitamin A ester has E. M. at a temperature of about 90°C higher than free vitamin A, and β-carotene has an E. M. at a temperature about 30°C higher.

E. M. of kitol itself, comparable to that of vitamin A, could not be observed but from the fact that its molecular weight is twice that of vitamin A, it can be assumed that it has an E. M. somewhat near that of β-carotene. However, kitol decomposes into vitamin A with heat so that to what extent the transformation into vitamin A took place during the analytical distillation still remains a problem.

Experiment 3. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil.

(1) The whale liver oil sample was obtained during whaling in the Antarctic Ocean and was informed as blue whale liver oil. Its general

characteristic is as follows:

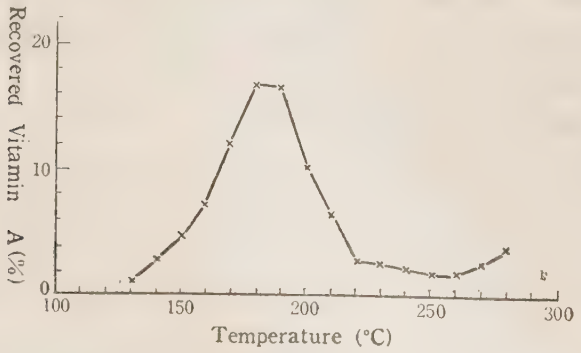
Specific gravity d_4^{15} 0.9230, refractive index n_D^{15} 1.4842, acid value 0, saponification value 166, unsaponifiable matter 11.5%, liver oil unit 312.

Unsaponifiable matter was obtained from 50g. of this liver oil and 80g. of oil of liver oil unit 66 was obtained by adding 35g. of C. Y. O. and 35g. of R. O., the same as before and analytical distillation carried on under the same condition as before. (Table 2 and Fig. 2)

Table 2. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil (1)

Number	Temp. (°C)	Distillate (g)	Liver Oil Unit	Liver Oil Unit × Weight	% of Recovered Vitamin A
1	130	1.3	38	50	1.0
2	140	2.2	62	136	2.6
3	150	2.2	111	242	4.6
4	160	1.8	212	382	9.3
5	170	2.2	297	651	12.4
6	180	2.6	341	885	16.8
7	190	2.6	333	865	16.5
8	200	2.7	205	552	10.5
9	210	2.6	179	358	6.3
10	220	1.5	106	159	3.0
11	230	2.4	59	140	2.7
12	240	1.7	69	117	2.2
13	250	1.3	72	94	1.8
14	260	1.3	78	100	1.9
15	270	1.7	75	127	2.4
16	280	2.1	95	200	3.8

Fig. 2. Analytical Distillation Curve of Unsaponifiable Matter in Whale Liver Oil (1)



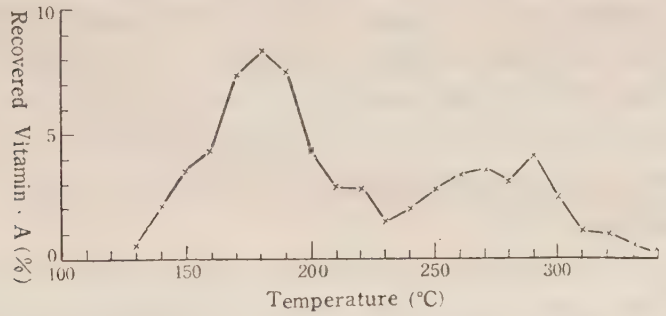
(2) 20 g. of new sample of the same liver oil as before was taken, unsaponifiable matter obtained from this, and to 240 g. of oil (liver oil unit 188) obtained by adding 35 g. of C. Y. O., 35 g. of R. O. and a further 60 g. of R. O. (C. Y. O. above 280°C) and 70 g of molecular distillation residue of

oil-shark liver oil and analytical distillation carried on (Table 3 and Fig. 3).

Table 3. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil (2)

Number	Temp. (°C)	Distillate (g)	Liver Oil Unit	Liver Oil Unit × Weight	% of Recovered Vitamin A
1	130	0.4	53	19	0.5
2	140	1.2	67	78	2.1
3	150	2.3	61	136	3.6
4	160	1.6	103	162	4.3
5	170	1.9	140	270	7.4
6	180	2.3	138	319	8.4
7	190	2.6	110	284	7.5
8	200	2.8	57	162	4.3
9	210	2.0	55	110	2.9
10	220	3.2	33	105	2.8
11	230	3.4	17	58	1.5
12	240	3.6	21	74	2.0
13	250	4.8	22	105	2.8
14	260	8.2	30	128	3.4
15	270	5.0	28	141	3.7
16	280	3.6	34	122	3.2
17	290	6.4	25	159	4.2
18	300	5.2	19	99	2.6
19	310	5.4	9	46	1.2
20	320	5.9	6	36	1.0
21	330	6.3	3	17	0.5
22	340	7.2	2	11	0.3

Fig. 3. Analytical distillation curve of unsaponifiable matter in whale liver oil (2)



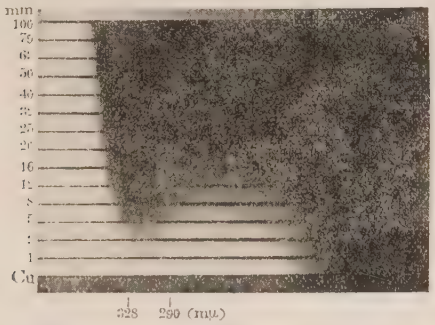
4. Absorption Spectrum of Analytical Distillate of Unsaponifiable Matter in Whale Liver Oil.

As a means of accertaining whether or not the two E. M. 's obtained as a result of analytical distillation of unsaponifiable matter in whale liver oil are due to vitamin A and kitol, absorprion spectrum of its E. M. distillate was taken. As a result of this, the first E. M. distillate showed a

typical vitamin A absorpition (Fig. 4) but in the second E. M. distillate, absorption was between the maximum absorption of vitamin A, 328 mμ, to the maximum absorption of kitol, 290 mμ, and somewhat stronger on the 290 mμ side (Fig. 5). An explanation for this may be that kitol was present in this whale liver oil and a part of it was decomposed by heat during analytical distillation. (Fig. 4 and 5).

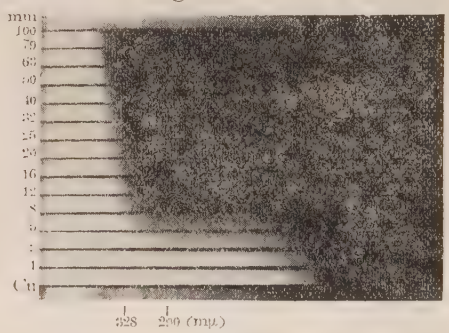
Experiment 4. Absorption Spectrum of the Distillate of Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil.

Fig. 4



Molecular Distillate at 180°C of Whale Liver Oil Unsaponifiable.

Fig. 5



Molecular Distillate at 280°C of Whale Liver Oil Unsaponifiable.

From the various distillations of (2) of Experiment 3, distillate of two E. M., 180°C and 280°C were taken as representative ones, a fixed quantity of the unsaponifiable matter after saponification was extracted with ether and absorption spectrum of this ether solution photographed. Concentrations appropriate for vitamin A in the 180°C distillate was 1:20,000 mol and 175,000 mol for the 280°C distillate. In the former, absorption appeared at 345, 328, 310, 300 and 290 mμ, being the strongest. In the latter, many vague absorption appeared from 328 mμ to 290 mμ and it was noticed that it was somewhat stronger on the 290 mμ than on the 328 mμ side.

5. Conclusion.

As a result of molecular distillation of unsaponifiable matter in whale liver oil, it was observed that the elimination maxima appeared at two places, 185°C and 270—290°C. The first maximum coincided with the maximum of unsaponiable matter in tunny liver oil and is ordinary vitamin A. The second, as observed from the absorption spectrum, absorption occured from 290 mμ, the absorption maximum of kitol, to 328 mμ, absorption maximum of vitamin A. Therefore, it is conculuded that kitol is present in whale liver oil and a part of it decomposed by heat to vitamin A

during analytical distillation.

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Molecular Distillation of Sperm Whale Blubber Oil

Seiichi Ishikawa, Yoshimori Omote, Hideo Kanno

1. Introduction.

Tsujimoto and Koyanagi[1] report that when sperm whale blubber oil was distilled in vacuum, only 60.25% of distilled oil was obtained at a reduced pressure of 2 mm. and distilled up to 295°C, and a greater part of the glycerides remained as residue. The authors undertook this research with the presumption that it will be possible to distill a greater part of the sperm whale blubber oil by molecular distillation, and the relation between temperature and quantity of distillate was examined, together with the general characteristic of each fraction. Also, quantitative analysis of each fraction was carried on. It was of great interest to find out to what extent cholesterol, which is present in minute quantities in this whale blubber oil, is contained in each fraction, which is related with analytical distillation of vitamin D.

2. Molecular Distillation with Pot Still.

At first, molecular distillation was carried out with pot still with the object of observing the general conditions such as the extent glycerides will distill over by molecular distillation. The result of this shows that 74.7% distilled over at 130—269°C. The characteristic and cholesterol content of each fraction is as shown in Table 1.

Experiment 1. Molecular Distillation with Pot Still.

The characteristic of the sperm whale blubber oil sample was as follows: specific gravity d_4^{15} 0.8599, refractive index n_D^{20} 1.4673, acid value 1.46, saponification value 158, iodine value 60.5, unsaponifiable matter 23.5%, cholesterol content 0.15% (0.12% in free state and 0.03% as ester).

40 g of sample was used each time and distilled in a vacuum of 10^{-3}

Table 1. Molecular Distillation with Pot Still

Fraction	Temperature Range (°C)	Quantity of Distillate (g)	Quantity of Distillate (%)	Refractive Index n_D^{20}	Acid Value	Saponification Value	Iodine Value	Cholesterol %
1	130—215	6.5	16.5	1.4645	2.74	95.5	52.4	19.0
2	215—225	12.9	32.4	1.4654	0.91	100.3	54.7	20.5
3	225—245	8.5	21.2	1.4674	0.87	132.4	63.8	12.4
4	245—269	1.7	4.2	1.4697	15.34	145.3	77.0	4.1
Residue	269	10.1	25.3	1.4735	1.57	224.2	64.4	44.0

mm. The average of each trial is shown in Table 1. The temperature was measured in the still. Micro-colorimetric method using Liebermann-Burchard color reaction was used for quantitative analysis of cholesterol in each fraction.

3. Molecular Distillation with Cyclic Still.

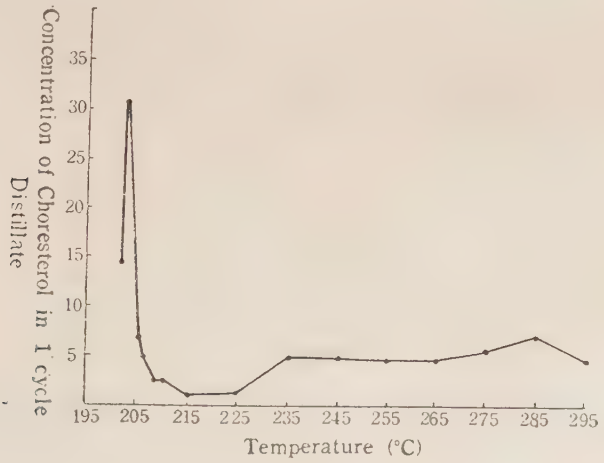
Next distillation was carried on with falling-film type cyclic still and 17 fractions were obtained between 195—295°C. Quantity of distillate was 90 % of the sample, and a greater part of the distillate was obtained at 205°C, about 60% of the sample and about 75% of the distillate. The characteristic and cholesterol content of each fraction are summarized in Table 2.

On examining the result indicated in Table 2, with reference to Toyama and Tsuchiya's[2] report on the composition of sperm whale blubber oil, it is concluded that higher alcohol ester was the principal component of the distillate at a temperature below fraction 12, reached the maximum at 205°C and glycerides distilled over at higher temperatures (See Fig. 1).

Furthermore, observations were made of fraction No. 5 as a representative of the 205°C fraction and it was shown that the fraction was a mixture of oleyl oleate and cetyl oleate, its mol-ratio being 3 : 2.

Also, the distillation of cholesterol was at the maximum from the first distillate to the 205°C fraction, its concentration increasing again near the end of the distillation. (See Fig. 2). It is assumed that the former is cholesterol in the free state and the latter, cholesterol in ester form. The presence of cholesterol in the free state and as an ester was also verified by quantitative separation analysis using digitonin.

Fig. 1. Relation Between Temperature and Quantity of Distillate



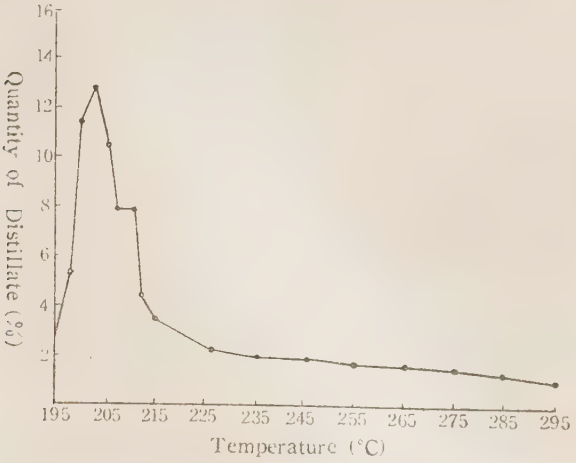
Experiment 2. Molecular Distillation with Cyclic Still.

285 g of sample was used, vacuum of 10^{-3} mm., temperature determination made at the central part of the heating oil bath within the evaporating column, and about 30 minutes were required for one cycle. Experimental result is summarized in Table 2.

Table 2. Molecular Distillation with Cyclic Still

Fraction No.	Tempt (°C)	Number of Cycle	Quantity of Distillation (g)	Quantity of Distillation (%)	Refractive Index n_D^{17}	Acid Value	Saponification Value	Iodine Value	Cholesterol %
1	195	2	6.9	2.4	1.4700	2.36	94.5	49.4	—
2	205	1	15.2	5.3	1.4678	1.06	121.8	53.6	14.6
3	205	1	31.8	11.8	1.4661	1.32	123.5	41.5	30.5
4	205	2	35.3	12.8	1.4668	0.78	136.1	64.9	6.6
5	205	2	29.1	10.4	1.4662	1.05	129.0	54.9	4.6
6	205	2	22.4	7.9	1.4666	1.10	128.2	64.6	3.5
7	205	2	22.7	7.9	1.4070	0.98	134.0	66.8	2.1
8	205	2	12.5	4.4	1.4670	1.10	125.1	67.5	2.1
9	215	2	10.1	3.5	1.4680	0.74	131.6	69.7	0.8
10	225	1	6.8	2.4	1.4680	1.69	126.5	78.1	1.1
11	235	1	6.2	2.0	1.4680	4.09	160.0	78.3	3.6
12	245	1	5.3	1.9	1.4680	4.82	151.8	76.9	4.6
13	255	1	4.8	1.7	1.4707	2.56	211.4	72.5	4.6
14	265	1	4.8	1.7	1.4710	2.44	216.4	69.1	4.6
15	275	1	4.5	1.6	1.4713	3.60	215.0	67.9	5.3
16	285	1	4.1	1.4	1.4724	2.44	221.6	67.2	6.5
17	295	1	3.1	1.1	1.4726	1.72	223.8	66.6	4.1

Fig. 2. Relation between Temperature and Quantity of Cholesterol Distillation



Experiment 3. Confirmation of Composition of Fraction No. 5.

5.4 g of Fraction No. 5 of Table 2 was used, unsaponifiable matter

extracted with ether after saponification, and from this a light yellow, semi-solid was obtained. This substance had an iodine value of 56.4, and referring to the report of Koyanagi and Tsujimoto[1], its ratio, assuming that it is a mixture of cetyl alcohol and oleyl alcohol, was 2 : 3. Also an yellow oil was obtained from the saponifiable matter by ether extraction after acidifiying with hydrochloric acid. The acid value of this oil was 187.8, iodine value 92.0 (theoretical value of oleic acid, acid value 190, iodine value 90) so it was confirmed that this oil was oleic acid.

4. Conclusion.

(1) By using pot still, about 80% of distillate was obtained between 195—295°C by molecular distillation of sperm whale blubber oil. Its principal component, about 75% of the entire distillate distilled over at 205°C and its components were confirmed as being oleyl oleate and cetyl oleate.

(2) Quantitative analysis of cholesterol in each fraction was carried out and it was possible to observe the presence of two maximum concentrations, which corresponds to the free state and ester state.

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Molecular Distillation of Fin Whale Liver oil

Aiji Kaneko

Introduction.

The author carried on molecular distillation of fin whale liver oil, examined the distillate with special emphasis on vitamin A and tried to determine its characteristics. That is, it has been pointed out that although whale liver oil shows a vitamin A color reaction it has little biological effect. Nakamiya[1] explained it with absorption spectrum and has reported that the peak has moved from 328 mμ. Furthermore, N. D. Embree[2] reports that the peak of absorption spectrum exist at four places and called it cyclized vitamin A. It has already been discovered that this does not have any effect in accelerating growth. The absorption spectra of these are very similar to those of vitamin A itself, and another important fact is that, at 200°C, vitamin A is transformed into cyclised vitamin A type due to the presence of an unsaturated acid such as oleic acid. Therefore, it is assumed that vitamin A is cyclized by unsaturated acid in the liver oil and thus lose its growth effect. A substance called kitol was discovered recently (3), kitol, was obtained from the unsaponifiable matter in whale liver oil by molecular distillation and crystalyzing was successful (4) but its structure was not determined, although experimentally, it is just two times that of vitamin A. It resembles carotene very much and is $C_{40}H_{60}O_2$, the peak of absorption being 286 mμ. The kitol also does not possess growth accelerating effect but its color reaction is the same as that of vitamin A.

In order to solve these problems, experiment was carried on as an attempt to discover cyclized viatmin A other than vitamin A coloring substance was concentrated by molecular distillation, following which, attempts were made to obtain crystals of these, but as explained later, obtaining pure crys. tals was not successful.

No. 1. Molecular distillation of fin whale liver oil.

As explained in the introduction, molecular distillation of liver oil was carried on, but distillation of the sample was carried on as it is, without pre-treatment. That is, the object was to detect the range through which vitamin A will distil and, at the same time, to find out to what extent

molecular distillation can be used as a possible means of refining oils and fats industrially.

Molecular distillation was carried on with samples which was not deodorized or neutralized in order to determine its deodorizing and neutralizing capacity. It was intended to crystalyze the concentrated vitamin A distillate by the Hamano Method[4].

1. Sample.

Sample used was Antarctic fin whale liver oil obtained from fin whale caught by the Taiyo Fishery Co., Ltd., in 1946 and its chemical constants are as follows:

Appearance (15°C)	Yellowish white, semi-solid
Refractive Index (18°C)	1.4950
Saponification Value	178.0
Vitamin A (C. L. O. U.)	50
Iodine Value (Wijs)	141.0
Unsaponifiable matter	1.7%

Also, the vitamin A determination during the experiment was made by the Oshima method[6].

2. Apparatus.

Molecular distillation apparatus used by the author was of all-glass type and its capacity is 1.5 kg., the area of the evaporation surface is about 200 cm^2, the distance to the condensation surface 5 mm and speed of circulation is 10 c.c. per minute. Pressure in the apparatus was determined by Geisler tube and was about 10^{-2}mm Hg.

For heating, parafin oil was placed in the cylinder of the evaporation surface and heated electrically, and the distilling temperature was the temperature of the bath so it is somewhat higher than the true temperature of the oil.

3. Experimental result and observation.

600 g of the liver oil sample was charged into the above apparatus, degasified at room temperatures, and further degasified after about two hours while carrying on circulation.

The circulation was carried on while working the diffusion pump and degasification continued, temperature raised gradually and distilled. As the liver oil sample was not saponified, the distillation temperatnre was generally high and the first distillate began at 220°C. Temperature range

was divided into four parts. Chemical constants of the distillates were as follows: General Characteristic of distillates of fin whale liver oil by molecular distillation.

Distillate	Distillation Temperature (°C/10^{-2} mm Hg)	Quantity of Distillate (g)	(%)	Appearance (15°C)	n_D^{18}	Acid Value	Iodine Value	Unsaponifiable matter (%)	V. A. (C. L. O. U.)	Order
Sample		600		Yellowish white semi-solid	1.4950	3.0	141.0	1.7	50	++
(1)	—220	3	0.5	Red liquid	1.5180	43.6	124.5	5.8	133	+++ +++
(2)	220—260	32	5.3	Golden color liquid	1.5168	29.0	115.0	6.1	800	+++
(3)	260—290	64	10.7	Yellow white semi-solid	1.4995	2.1	97.5	2.8	154	+++
(4)	280—300	95	15.8	Yellow white semi-solid	1.4895	1.5	94.5	9.8	50	++
Residue		395		Light brown liquid	1.4930	1.1	130.7	1.4	3	+

In the above table, in regards to odor, it was fortunate that the original sample had considerable odor and so it was used for determining the odor of each distillate tested on eight healthy girls. Needless to say, it is difficult to indicate odor quantitatively and the result is based on general smelling senes.

Odor of residue oil was very slight, acid value low, little polymerization and it was observed that is was suitable for edible use.

Vitamin A was concentrated into this fraction. Therefore, if liver oil which has been deodorized by pre-treatment was used, it can be used as vitamin A for addition in margarine.

A greater part of the free fatty acid was distilled over at 220°C.

4. Summary.

The following conclusion was drawn from the above molecular distillation, from the point of view of refining oils and fats:

(1) Sample should be completely dehydrated and degasified.

(2) In regards to apparatus, the pump should be powerfull and circulating apparatus be absolutely secure.

(3) Neutralization is almost complete by molecular distillation.

(4) It is possible to obtain concentrated solution of vitamin A 16 times that of the sample, and if it has been deodorized by pre-treatment, it is possible to use it for addition to margarine.

(5) Odor will accumulate to the same fraction as vitamin A but since it will distill over in the second fraction after neutralization, it is believed

that the molecular weight is somewhat larger than the free acid.

(6) There is little polymerization of the residue oil so it is suiable as edible oil.

No. 2. Derivation vitamin A crystals.

Attempts were made to derive crystals from the vitamin A distillate concentrated by the method described in the previous report, by the Hamano Method[5], but pure crystals could not be obtained because the quantity of the starting sample was too small. In Hamano Method, anhydrous maleic acid is made to condense directly on vitamin A ester and crystals obtained. Next it is saponified and the acid portion of its ester is also determined.

30 g of the above concentrated liver oil is dissolved in 100 g of anhydrous benzol and anhydrous maleic acid is dissolved at the same time, carbon dioxide gas is sealed into the tube and made to react by heating at 100°C. After 48 hours, Carr-Price reaction is absolutely absent so it is assumed that condensation was completed at this point. In the inner surface of the sealed tube, a small quantity of white, amorphous crystals appeared.

After the reaction, benzol was distilled off at reduced pressure, the viscous liquid poured into 3 liters of anhydrous petroleum ether and allowed to crystalize. After standing for one day, about 0.48 g of white, powderlike crystals, were obtained at the bottom of the container. The melting point of this crystal was 189°C and since recrystalization could not be obtained at 220°C, as referred to in reference (5) it was continued to the next procedure. That is 0.48 g of the crystal was saponified with 10 c.c. of 0.1 *N* $KOH \cdot C_2H_5OH$ and acidified with HCl. At this stage, only oily substance was freed and crystalline substance could not be obtained. This oily substance was extracted with anhydrous petroleum ether and the soluble part was a white crystal with a melting point of 37°C. This is the acid portion of vitamin A, but since the quantity of the sample was too small to confirm it, it was discontinued.

The oily substance insoluble in petroleum ether was recrytalized three times with benzol-ether, but the melting points were not constant. That is, it was only observed that it began to melt at 160°C, become a brown viscous substance in the vicinity of 220°C and finished melting into reddish brown at 280°C. This is very similar so the behavior of anhydrous maleic acid mixture of carotene referred to in Nakamiya's thesis[7] observation of the experimental result.

(1) The derivation of vitamin A crystals was by Hamano's method.

(2) Anhydrous maleic acid additive was obtained, but on acidifying with HCl, only an oily substance was produced and no crystals were obtained.

(3) The melting point of the sbustance, assumed to be the petroleum ether soluble acid component, was 37°C.

(4) The petroleum ether soluble portion was recrystalized with C_6H_6·$(C_2H_5)_2O$ but the melting point was not clear and it was observed that the behavior resembled that of anhydrous maleic acid mixture of carotene.

As indicated above, the research on crystal derivation by anhydrous maleic acid mixture was unsuccessful, but it is concluded that besides vitamin A, a higher molecular substance resembling carotene, is also present in whale liver oil. Because of this, it was thought that it did not crystalize out but only formed an oily substance and also, the melting point was not clear. At present other methods for determining this is being studied.

The above reserch was carried on in the chemistry laboratory of the Tokyo University of Literature and Science and gratitude is expressed to Dr. Ishikawa, Dr. Mitsuwa and Omote, master of science, for their instruction. Also, deep appreciation is expressed to the Taiyo Fishery Co., Ltd., and Sakuma, Manager of plant, for supplying the sample.

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Determination of Tryptophane in Whale Meat

Shichiro Akiya, Keiichi Takahashi

Since the discovery in 1906 by Rose, Willock, and Abderhalden, that tryptophane was an indispensable amino acid in human body, many reports have been published regarding its change and mechanism in the living body. The authors, in order to determine the content of this amino acid in whale meat and to determine whether whale meat could be utilized as resource for peptone production, carried out comparative tests on quantitative determination of tryptophane and thereby found a novel revised method.

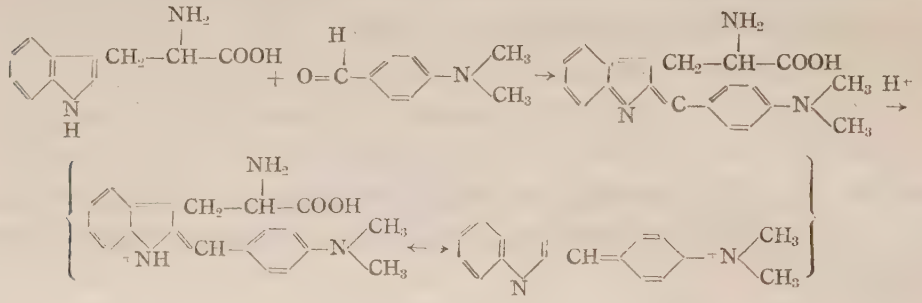
Theoretical

There are already numerous methods of determining tryptophane, which, unlike other amino acids, possesses indole nucleus in its structure so that there are qualitative, quantitative and colorimetric methods for its determination. Homer[1] led tryptophane to its bromo compound and then titrated excess amount of bromine by iodometry. Homer maintains that tryptophane gives octabromo-tryptophane by bromination. However, Levner says that it forms tribromo compound and this point has not been settled as yet. If this point can be settled, bromination methhod is excellent and simple because the reaction by halogen is limited to free tryptophane except ablin (N-methyltryptophane). All other tryptophane derivatives and addition products do not react to this reagent.

Quantitative determination by Hopkins-Cole reagent of mercury-tryptophane method and its modification by Onslon[2] by the determination of N-value in mercury-tryptophane are some of other methods but the most ordinary method is to use colorimetric measurement. Colorimetric reagent employs, chiefly, glyoxylic acid, formaldehyde and p-dimethylaminobenzaldehyde, which possess carboxyl radical. Others employ xanthoprotein[3] and Pauly's diazo reactions but these are also positive to amino acids having benzene ring, such as tyrosine and phenylalanine, so that a more complicated processes are required and cannot, moreover, be taken as a specific reaction for tryptophane. It follows, therefore, that colorimetric determi-

nation of tryptophane should employ reagents possessing carboxyl radical, especially the Ehrich reagent recommend by Neuber and Rohde, Hohm[4], May and Rose[5].

Ehrlich reagent employs p-dimethylaminobenzaldehyde which is condensed to tryptophane in H_2SO_4-acidity and the dyestuff is thus formed. Chemical mechanism of the dyestuff formation can be explained as follows:



Colouring

The above reaction necessitates the presence of H^+ so that, if OH^- is present, the reagent aldehyde does not condense to the nucleus but with the amino radical in the side chain. Ehrlich reagent, therefore, colors only under strong acidity.

If tryptopnane itself undergoes change only under this condition, this reaction would become unsuitable for the determination of tryptophane. The authors, therefore, observed the change in tryptophane, with considerations of the effects of reaction time and temperature, and found, as is generally known, that tryptophane resisted acids. In other words, no change could be observed when tryptophane was boiled in aqueous solution of mineral acids. However, when carbohydrates, beside tryptophane were present, these reacted under strong acidity and formed the so-called Fumine substance of unknown structure. In these cases, there is no sense in colorimetric determination of tryptophane. However, if this reaction is carried out under 40°C, Fumine substance is not formed, even under strong acidity. This leaves the question of reaction time. As a result of determining minimum time required for total tryptophane in protein to be isolted by hydrolys it was found that, although it differed slightly according to the proteins used, the hydrolysis was completed in 5—10 days with proteins from whale meat and casein. Side reactions occurred when the reaction time was prolonged over two weeks and the amount of tryptophane found was decreased considerably. These experiments were conducted under the

presence of p-dimethylaminobenzaldehyde. The reason for this is that, even if carbohydrates are isolated from proteins by hydrolysis, tryptophane will have condensed with p-dimethylaminobenzaldehyde before it had time to bond with carbohydrates to form Fumine substances. Rose determined the amount of tryptophane in protein by using Ehrlich reagent and 20% HCl, with the reaction at 35—37° for 24 hours and at room temperature for 48 hours, determining the coloration here formed. The authors learned that, uder these conditions, complete hydrolyses of protein cannot be expected and therfore, determineed the time where coloration by Ehrlich reagent was at its maximum by determing amino form nitrogen by Van Slyke method by which the degree of protein hydrolysis would be measured. As a result, the afore-mentioned reaction time of 5—10 days, temperature of 35°C, and degree of protein hydrolysis of 85—90% were obtained.

Reaction above these figures would give discoloration which eventually gave decreased amount of tryptophane.

Results obtained by Boyd[6], Hohm and Konm[7], on pure tryptophane coincided with the results obtained by the authors.

Experimental

Colorimetric Standard and Test Solution:

Preparation of Standard Solution: For the preparation of this solution, Protiens not containing tryptophane, such as gelatine or zein, are used. 3a (a=50 mg) of this protein is added to 1 mg of pure tryptophane.

Test Solution: (1—2) a of sample protein is added to (1—2) a of gelatine bringing the amount of total protein to 3a. The object of the addition of gelatine is to shorten reaction time somewhat and to control the coloration.

Method:

100 g of the sample protein and 50 mg of gelatine are placed in 100c.c. measuring flask. On the other hand, standard substance is prepared by placing 150 mg of gelatine and a determined quantity of pure trytophane in 100 c.c. measuring flask. 1 c.c. of Ehrlich reagent (5% p-dimethylamino-benzaldehyde in 10% H_2SO_4 solution) is added to each of the above flasks, shaken well, and bring the amount of solution to 100 c.c. by adding 20% HCl. This is left in a thermostat at 35°C and the coloration observed every day. Colorimetric determination with Dubosqui's colorimeter is made when the coloration seems to be at a maximum. In the present experiment, the

reaction required 5—10 days. Following are the results obtained:

Content of tryptophane in whale meat.

After dehydration of whale meat by acetone, degreasing and drying, the sample is taken of this dried whale meat. Crude protein contained in the sample was 66.3% as determined by Kjeldahl method. Content of tryptophane in whale meat.

Sample	Tryptophane (%)	Average (%)
A	0.97	
B	0.91	0.95
C	0.96	
D	0.94	

Content of Tryptophane in Casein not purified.

Sample	Tryptophan (%)	Average (%)
A	1.66	
B	1.83	1.79
C	1.89	

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Protein digestive power of Sperm whale Pancreatic Enzyme

Yasutaro Ishikawa, Setsuzo Tejima

The feeding habit of cetacea is very peculiar, that is, they do not take any carbohydrates but depends entirely on protein food.

These may have some special digestive enzymes and the authors' attention were attracted by pancreatic enzyme and anticipated that this may be a strong protease.

Also cetacea eats live feed so that the digestive enzyme the feed posses may be utilized in the alimentary tract. If this is considered, the digestive enzyme excreted by whales may be only a small quantity.

If they are strong as first anticipated, there may be some industrial use for them because the quantity of whale pancreas is very large.

At any rate, it is necessary to ascertain the protein digestive power of pancreatic enzyme. Fortunately, the authors were able to obtain pancreas from two sperm whales at the Ayukawa whaling ground and experiment was carried on with these.

Both of the sperm whale were about 40 ft. long and they were not fresh because 30 hours elapsed from the time they were caught to the time when they were dissected.

After dissection, the pancreas was removed, fatty tissues, connective tissues and other unnecesary parts were removed, as much as possible, placed in cans immediately frozen and brought to the laboratory. On the way, special attention was paid so that the temperature did not rise. This was well minced with a meat mincer, mixed and stirred until uniform, stored in a refrigerator and taken out as the experiment required.

Determination of digestive power was carried on along the pancreatin method described in the Japan Pharmacopoeia. That is, 0.2% casein solution was used as substrate, extracted solution obtained by extracting a fixed quantity of minced pancreas with fixed quantity of water, was added to 5 c.c. of this in stages, water was added until the total content became 10 c.c. This was made to react for one hour at 40°C, 3 drops of a solution composed of 10 c.c. of alcohol, 1 c.c. of glacial acetic and 9c.c. of water was

added to this digestion solution and the presence of slight tutbidity was taken as the end of digestion.

Preparing of casein solution is as follows that is, refined casein by Hammersten's method, is dried in a H_2SO_4 desiccator, 2 g. of this is removed and well dispersed in about 50 c.c. of water. To this is added 2 c.c. of 0.1 *N* KOH, shaken to dissolve and made up to 100 c.c. with water after it has dissolved.

Experimental objects were to determine, the digestive power of fresh pancreas (those which went trough the above procedure), the relation between time and activition of digestive power when it was autolysed at 40°C in neutral reaction and the same relation in autolysis at 40°C when sligtly acidic (initial pH 5.4) and the digestive power when minced pancreas was dried with acetone and pulverized.

Furthemore, the same experiment was carried on with cow pancreas and the digestive powers of cow pancreas and whale pancreas were compared.

Experiment

1. Protein digestive power of fresh pancreas.

Enzyme solution made by dispersing 1 g. of minced pancreas in 100 c.c. of water is filtered with fluted filter paper. The analysis of this minced panccreas is 73.7% moisture and, 26.3% dried matter but this dried matter contained considerable quantity of fat. Therefore, 100 c.c. of this extraction liquid may be equivalent to ca. 0.2 g. of dried pancreas so that in such an estimation method, 0.2 g. is assumed to be not such a discrepancy. By doing this, it will be simple to calculate the unit quantity of pancreas which digested so many times casein because the casein solution is 0.2 g./100 c.c. All experiments hereinafter were carried on by this method.

Experimental result is as shown in Table I and this pancreas did not have any digesting power.

Table I. Digesting Power of Fresh Pancrea

No.	0.2% Casein solution c.c.	1 g./100 c.c. pancreas extraction liquid c.c.	Water c.c.	Ratio of casein pancreas dried matter	Turbidity
1	0	5	5	0	±
2	5	5	0	1	+
3	5	2	3	2.5	+
4	5	0.5	4.5	10	+

In turbidity, — indicates transparent, ± slight turbidity, and turbid or precipitation is indicated by +. This applies to all following experiments.

2. Increase of digestive power by neutral autolysis.

Minced pancreas is placed in a conical flask, a small amount of toluol added and sealed. This is placed in a thermostatic bath, of 40°C taken out at the time indicated in Table II and digestive power determined. Pancreas extraction liquid is made in the same manner as before, that is, 1g. of pancreas is extracted with 100 c.c. of water and filtered.

Table II. Increase of digestive power by autolysis

Hours left standing	No.	0.2% Casein Solution c.c.	1g./100 c.c. Pancrea extraction liquid c.c.	Water c.c.	Ratio casein pancreas dried matter	Turbidity
3.5 hours	1	0	5	3	0	—
	2	5	5	0	1	± (digested)
	3	5	2	5	2.5	+
10.5 hours	1	0	5	5	0	—
	2	5	5	0	1	—
	3	5	2	3	2.5	± (digested)
	4	5	1	4	5	+
24 hours	1	0	5	5	0	—
	2	5	5	0	1	—
	3	5	2	3	2.5	— (digested)
	4	5	1	4	5	+
	5	5	0.5	4.5	10	+
48 hours	1	0	5	5	0	—
	2	5	5	0	1	— (digested)
	3	5	2	3	2.5	+
	4	5	1	4	5	+
	5	5	0.5	4.5	10	+
	6	5	0.2	4.8	2.5	+

As can be seen from Table II, when it is allowed to autolyse at 40°C, digestive power increase somewhat, the maximum of increase being between 10—24 hours, and its maximum value being at 2.5. That is, pancreas digest 2.5 times its weight of casein.

3. Increase of digestive power by slightly acidic autolysis.

10 g. of minced pancreas is mixed with 2 c.c. of water and 2 c.c. of 0.1 *N* HCl, a small amount of toluol added, made into several samples, sealed and stored in a thermostatic bath of 40°C. One of these is taken out at each time indicated in Table III and digestive power determined. The initial pH of this mixture was 5.4.

The concentration of the enzyme solution is exactly the same as in the previous experiment and 100 c.c. of the enzyme solution is equivalent to 1 g. of the original pancreas (0.2 g. as dried matter).

Table III. Increase of digestive power by autolysis (Initial pH 5.4)

Hours left standing	pH at time of experiment	No.	0.2% Casein colution c.c.	1 g./100 c.c. pancreas txtracted soluion c.c.	Water c.c.	Ratio Casein pancreas dried matter	Turbidity
3 hours		1	5	5	0	1	± (digested)
		2	〃	2	3	2.5	+
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+
12 hours	5.6	1	5	5	0	1	−
		2	〃	2	3	2.5	− (digested)
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+
24 hours		1	5	5	0	1	−
		2	〃	2	3	2.5	± (digested)
		3	〃	1	4	5	+
36 hours		1	5	5	0	1	−
		2	〃	2	3	2.5	± (digested)
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+
48 hours	6.2—6.4	1	5	5	0	1	− (digested)
		2	〃	2	3	2.5	+
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+

As can be seen from the result, it is about the same in the case of slightly acidic as in the case of neutral, maximum increase being between 12—36 hours and its maximum value is the same, 2.5.

In order to show more readily the relation between hours left standnig and digestive power for neutral and slightly acidic, it is as shown in the Summary Table IV.

Table IV. Hours of autolysis and digesting power.

Neutral, left standing at 40°C		pH 5.4, left standing at 40°C	
Hours left standing	Digesting power casein/panereas	Hours left standing	Digesting power casein/pancreas
0	0		
3.5	1	3	1
10.5	2.5	12	2.5
24	2.5	24	2.5
		36	2.5
48	1	48	1

5. Increase of digestive power of cow pancreas by autolysis.

Cow pancreas was obtained directly after slaughtering from the Shibaura Slaughter House. This was frozen at −35°C, minced and used in experiment, Temperature of autolysis and other experimental conditions are exactly the same as in the case of whale.

The result is as shown in Table V. As can be seen from Table V.,

casein digested when fresh is 10 times and is 25 times casein after it is autolysed for 24 hours at 40°C.

In Table V. the standard concentration of enzyme solution was 1 g. of minced pancreas extracted with 100 c.c. of water. When it is diluted and used, the concentration is shown in parenthesis.

Table V. Increase of digestive power of cow pancreas by autolysis. by autolysis

Hours left standing	No.	0.2% casein solution c.c.	Pancreas extracted solution c.c.	Water c.c.	Ratio **casein** pancreas dried matter	Turbidity
0 hour	1	5	5	0	1	±
	2	〃	2	3	2.5	〃
	3	〃	1	4	5	〃
	4	〃	5 (×1/10)	0	10	〃 (digested)
	5	〃	2 (〃)	3	25	+
	6	〃	1 (〃)	4	50	+
12 hours	1	5	5 (×1/10)	0	10	−
	2	〃	2 (〃)	3	25	± (digested)
	3	〃	1 (〃)	4	50	+
	4	〃	2.5 (×1/50)	2.5	100	〃
	5	〃	2 (〃)	3	125	〃
	6	〃	1 (〃)	4	250	〃
24 hours	1	5	5 (×1/10)	0	10	+ (digested)
	2	〃	2 (〃)	3	25	〃
	3	〃	1 (〃)	4	50	〃
	4	〃	2.5 (×1/50)	2.5	100	〃
	5	〃	2 (〃)	3	125	〃
	6	〃	1 (〃)	4	250	〃

5. Protein digestion of pulverized whale and cow pancreas dried matter.

Next, the authors prepared pulverized dried pancreas of whale and cow and determined the digestive power of both. That is, minced pancreas is left standing at room temperature (2—8°C) for one night, placed in a large quantity of acetone to dehydrate, filtered with suction and washed several time by pouring on it. Next, it is dried by aeration, well dried in a H_2SO_4 desiccator, pulverized in a morter, sieved with a 60 mesh sieve, and the digestive power of those which passed through the sieve was determined. Also, for comparison, the digestive power of both whale and cow pancreas before dring were determined (after standing for one night).

Result of determination and the detail of yield from minced pancreas to sieved powder is shown in Table VI and Table VII.

As can be observed from Table VI, the digestive power of whale pancreas before drying is 0, but that of cow is 50 times. For dried pulverized whale pancreas, it is 2.5 times, and 125 times for cow pancreas.

In table VI the standard for pancreas extracted solution for fresh pancreas, was 1 g 100 c.c. and 0.2 g 100 c.c. in the case of dried pulverized

pancreas, these being gradually diluted and used. Concentration is indicated in parethesis.

Table VI. Digestive power of dried pulverized whale and cow pancreas and before drying.

Kind	No.	0.2% Casein solution c.c.	Pancreas extracted liquid c.c.	Water c.c.	Ratio casein pancreas dried matter	Turbidity
Fresh whale pancreas	1	5	5 (1g./100 c.c.)	0	1	+
	2	〃	2 (〃)	3	2.5	+
	3	〃	1 (〃)	4	5	+
	4	〃	0.5 (〃)	4.5	10	+
Dried pulverized whale pancreas	1	5	5 (0.2 g/100 c.c.)	0	1	—
	2	〃	2 (〃)	3	2.5	± (digested)
	3	〃	1 (〃)	4	5	+
	4	〃	5 (〃 ×1/10)	0	10	+
	5	〃	2 (〃 ×1/10)	3	25	+
Fresh cow pancreas	1	5	5 (1 g/100 c.c.)	0	10	—
	2	〃	2 (〃)	3	25	—
	3	〃	1 (〃)	4	50	— (digested)
	4	〃	2.5 (〃 ×1/5)	2.5	100	+
Dried pulverized cow pancreas	1	5	2 (0. 2g.1000 c.c.)	3	25	—
	2	〃	1 (〃)	4	50	—
	3	〃	3.5 (〃 ×1/5)	1.5	70	—
	4	〃	2.5 (〃 ×1/5)	2.5	100	—
	5	〃	2 (〃 ×1/5)	3	125	± (digested)

Table VII. Yield on drying and sieving minced pancreas.

Minced pancreas g.		Acetone dried matter g.	Sieved matter g.	Sieved residue g.	Loss on sieving g.
Whale	99	15.1	9.6	5.3	0.2
Cow	49	9.8	8.5	1.1	0.2

Conclusion.

There is almost no digestive power in the fresh sperm whale pancreas which was used in the experiment and by autolysis was only 2—3 times casein. There is almost no change in progress of increase by autolysis in both neutral and slightly acidic condition.

In the case of cow pancreas, under the same condition, it is easy to obtain a digestive power of about 25 times by autolysis.

On standing for one night at room temperature, the digestive power of whale pancreas was 0 but that of cow pancreas was 50 times. Also, when these are dried and pulverized, digestive power of whale pancreas was 2.5 times and 125 times in case of cow pancreas.

In comparing whale and cow pancreas, the amount of connective tissues in the case of whale is very large and only a small part was pulverized.

Experiment on Digestion of Whale Meat by Koji-mould

Saburo Tsukamoto

Introduction.

It has already been reported (Akiya, Ishikawa, Tejima) that whale pancreas can be used for manufacturing peptone from whale meat and a product suitable for bacteria culture can be obtained by digesting whale meat with this enzyme.

However, the proportion of pancreas compared with the size of the whale is very small, 3—5 kg in the case of sperm whale of about 45 ft in length and 10 kg in the case of sei whale of about the same size. Also, the total quantity of whale pancreas in the country is very small so that the production of peptone is naturally limited by the quantity of pancreas. Furthermore, pancreas is an important raw material for pharmaceuticals such as protease, insulin which is the special medicine for diabetics, etc, and since there is much use for it in the future, if is necessary to find an enzyme source other than pancreas, for manufacturing peptone.

The authors' attention was turned to koji-mould (Aspergillus Oryzae), which is relatively easy to obtain and easy to manufacture, as a substitute for this pancreatin.

The reaction of koji-mould on protein, by which the protein is decomposed into amino acids by the protease, is widely used in various fermentation industry, but when whale meat is used as a source of protein and decomposed, peptone, peptide and amino acids are produced. The fact that peptone is suitable for bacteria culture by this method has already been explained (Tejima).

In carrying on this decomposition, there is a great difference in its enzymic action according to kind of koji-mould, kind of culture substrate, culture conditions, enzyme reaction conditions, and pretreatment conditions of whale meat, etc.

The object of this experiment is to find out these conditions. Protein will first become water soluble peptone by the reaction of enzyme so that the degree of decomposition at the beginning can be determined by the amount of water soluble portion.

Therefore, roughly, the quantity of peptone can be calculated by evaporating and drying the water soluble substance produced by decomposition of whale meat by koji-mould.

In this experiment, sweet potato flour and potato starch were chosen as culture substrate and whale meat broth was used for mixing in this. Ordinarily, rice is used for koji-mould culture, but the present condition in the country does not permit its use. Tejima, used soy bean meal but this is principally and imported goods so it was assumed to be rather undependable as raw material. Also, it is a known fact that the koji-mould will depend on the kind of enzyme produced by different substrate so that whale meat broth was mixed in it in order to produce as much protease. As explained later, the decomposition power was stronger the more whale meat broth used, as anticipated.

Experiment.

Koji-mould Culture.

As substrate, three types were used, one with sweet potato flour kneaded with equal amount of water, formed into pellets the size of soy bean and steamed in an oven, the second with sweet potato flour kneaded with half its quantity of water and half its quantity of whale meat broth, and the third with sweet potato flour kneaded with equal amount of whale meat broth. Whale meat broth referred to here is meat broth produced by decomposing finely chopped whale meat with heat. The object is to supplement the protein in sweet potato flour and also because it was assumed that it will strengthen the protease in the koji-mould.

As coditions for culture, the substrate steamed as above in cooled and koji-mould spores cultured with glutinous rice is shaken on it and experiment on rate if decomposition of whale meat experimented with those left standing for 1 day, 2 days and 3 days at 31—33°C.

In regard to propagation of koji-mould when whale meat broth is not added, only a slight formation of mycelium with te 1 day culture is observed, entirely covered with te 2 days culture and spores were well formed with 3 days culture, but when whale meat broth was added, it because more active, such that 1 day culture was entirely covered with mycelium and spores were entirely formed with 2 days culture.

Digestion of whale meat.

Digestion was caried on by the following method with koji-mould cultured by the above method. For each 5 g of sweet potato flour used for culture, 100 g of finely chopped frozen fin whale meat is boiled, filtered with a cloth, 100 c.c. of sterilized water is added to the insoluble matter, that is, meat protein, boiled to sterilize, koji-mould cultured by the above method and a small amount of toluol is added and preserved at 31—33°C.

Digestion time was divided into three stages, 48 hours, 74 hours and 120 hours. After digestion, this is filtered with suction, using cloth to separate the soluble and insoluble portions and each portion is evaporated, dried (105°C) and weighed.

Peptone was calculated by the following formula:

$$\text{Peptone (g)} = \left\{ \text{Residue (hydrous) (g)} - \text{dried residue (g)} \times \frac{\text{filtrate (g)}}{\text{dried filtrate (g)} + \text{dried filtrate (g)}} \right\}$$

Table 1.

Koji-mould culture time	No. of days digestion	Kind of culture	Filtrate (a)	Dried filtrate (b)	Residue (hydrous) (c)	Dried residue (d)	Peptone $\left(\left(c-(d)\times\frac{(a)}{(b)}\right)\times(b)\right)$	Remarks
No. 1 24 hours culture		A	99 g	4.5g	67 g	26 g	6.35 g	Substrate is 5 g sweet potato flour kneaded with 5 g water
	a (2 days)	B	89	5.2	71	29	7.6	Substrate used is 5 g sweet potato flour kneaded with 2.5 g water and 2.5 g meat extract
		C	94	5.1	78	25.5	8.0	Substrate used is 5 g sweet potato flour kneaded with 5 g meat extract
	b (3 days)	A	105	5.3	61	25	7.3	
		B	97	5.7	61	25	7.85	
		C	125	6.1	67	25	8.15	Water added to meat was too much
	c (5 days)	A	96	5.5	60	21.1	7.7	
		B	115 (113 c.c.)	6.0	62	20.2	8.2	
		C	85 (84 c.c.)	6.0	73	21.8	9.6	Filtering somewhat diffcult
No. 2 48 hours culture	a (2 days)	A	94	4.2	64	23	6.05	
		B	98	5.3	62	23	7.4	
		C	100	6.0	60	23	8.2	
	b (3 days)	A	96	4.9	63	22	7.0	
		B	84	5.0	70	22	7.85	
		C	89	5.7	66	21	8.6	
	c (5 days)	A	98	5.8	63	20.6	8.3	
		B	91	6.0	62	19.5	8.8	
		C	81 (79 c.c.)	6.2	69	19.5	10.0	

No. 3 72 hours culture	a (2 days)	A	100	6.1	59	20.7	8.4	Filtering somewhat difficult
		B	93 (90 c.c.)	6.0	65	16.9	9.1	
		C	97	6.6	60	16.9	9.5	
	b (3 days)	A	89 (87 c.c.)	6.1	64	21	9.0	Filtering somewhat difficult
		B	99	6.6	59	21	9.1	
		C	99	7.2	61	19	10.25	
	c (5 days)	A	91	6.0	73	19.1	10.0	Filtering somewhat difficult (c) had considerable moisture
		B	84	6.8	66	15.0	10.1	
		C	108	7.8	50	13.2	10.5	
	d (8 days)	D	—	—	—	—	11.2	

Experimental result is as indicated in Table 1.

As can be seen from the above table, digestion is greater when amount of whale meat broth added is greater and when the culture time is longer. Furthermore, digestion will be greater and more soluble matter obtained when digestion time is longer. However, if the digestion time is long, the undecomposed portion will become considerably less and the total of this and the soluble portion will be reduced. It is believed that this is due to the fact that the greater part of the soluble protein is further decomposed and gasified into ammonia gas, etc. Also change in quantity of soluble portion will be retarded when decomposition is continued further. That is, the culture time of koji-mould, in the condition of digestion, had the greatest effect and the effect of time in digestion increased greatly during the first 2 days and becomes very small after that.

In this experiment, 10.5 g of soluble portion was obtained from 100 g of frozen, fresh meat when decomposition was greatest.

The above digestion was carried on at 33°C, but next, digestion was carried on with sperm whale meat under the best conditions of the above experiment, changing the temperature to 37°C and 42°C, the previous experiment was carried on at pH of about 7, but dilute H_2SO_4 was added in this experiment in order to carry it out at pH 4.2.

Furthermore, as substrate potato starch was used instead of sweet potato flour.

The result is as indicated in Table 2.

Table 3 indicates the principal curves of Tables 1 and 2.

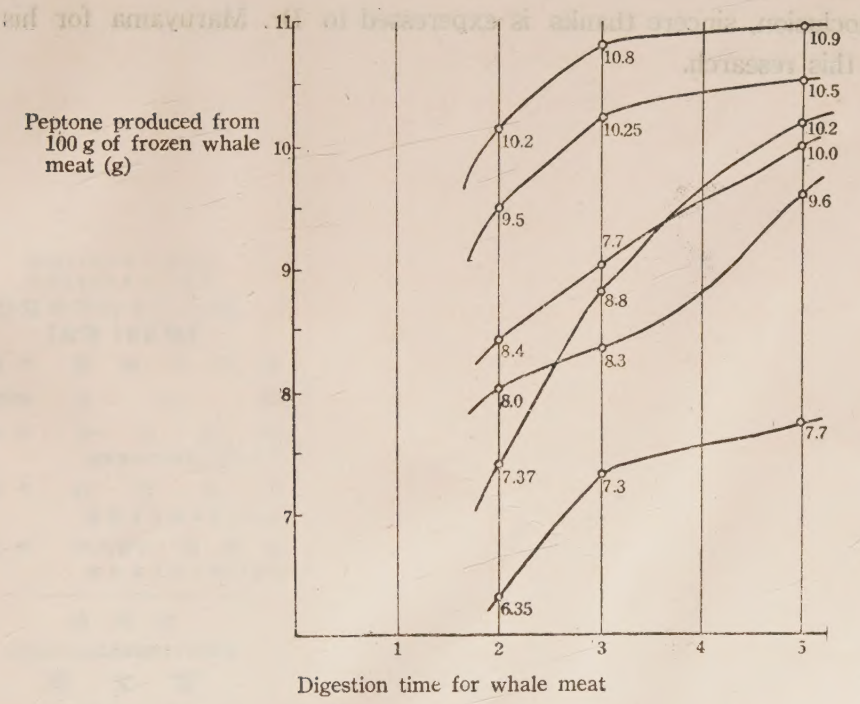
In this experiment, it was difficult to filter after digestion so the unreacted slimy matter was separated with a centifugal separator.

Table 2.

Decomposition temperature	Substrate for koji-mould culture	Digestion time	Filltrate	Dried filtrate	Slimy matter (hydrous)	Dried Slimy matter	Calculated peptone	Remarks
No. 1 37°C	Sweet potato flour +whale meat broth	a (2 days)	110 g	5.3 g	60 g	17.0 g	7.37 g	
		b (3 days)	112.3	6.2	60.8	13.3	8.82	
		c (5 days)	114.8	7.3	55	9.5	10.2	
	Potato starch + whale meat broth	d (5 days)	93.0	5.6	60.7	13.8	8.43	
	Sweet potato flour +whale meat broth	e (5 days)	115.7	6.9	66.2	20.0	9.6	
No. 2 42°C	Sweet potato flour +whale meat broth	a (2 days)	115.0	7.5	57	16.2	10.18	
		b (3 days)	120.9	7.6	57.0	12.7	10.8	
		c (5 days)	127.0	8.3	47.3	9.0	10.9	
	Potato starch + whale meat broth	d (5 days)	119.0	6.8	53.9	10.2	9.3	
	Sweet Potato flour +whale meat broth	e (5 days)	122.5	7.2	66.6	19.4	9.9	

(During digestion, 0.3 g of various oils and fats were obtained)

Table 3.



It can be observed from this table that maximum digestion is at 42°C, digestion is somewhat less at pH 4.2 than at pH 7 and less when potato

starch was used than when sweet potato flour was used. It is assumed that the koji-mould did not propagate fully into the interior because of the formation of gel in case of starch, and also, the culture method was somewhat difficult due to gel formation.

Conculsion.

The following conculsion is drawn from the above experiments. The best condition for producing peptone by digestion whale meat with sweet potato koji-mould, within the sphere of this experiment, are as follows:

1. To use a large quantity of whale meat broth with sweet potato flour in producing koji-mould, and to use as much time in culture.
2. Digestion temperature is about 42°C.
3. Digestion time is about 5 days.
4. Care must be taken to prevent abnormal fermentation.

About 10% of peptone can be obtained from frozen whale meat, and since the protein content of frozen whale meat is about 20%, about 50% is decomposed.

In cnoclusion, sincere thanks is experessed to Dr. Maruyama for his advice in this research.

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